

## RESEARCH ARTICLE

# Xanthohumol, a chalcon derived from hops, inhibits hepatic inflammation and fibrosis

Christoph Dorn<sup>1</sup>, Birgit Kraus<sup>2</sup>, Magdalena Motyl<sup>2</sup>, Thomas S. Weiss<sup>3</sup>, Manfred Gehrig<sup>4</sup>, Jürgen Schölmerich<sup>1</sup>, Jörg Heilmann<sup>2</sup> and Claus Hellerbrand<sup>1</sup>

<sup>1</sup>Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany

<sup>2</sup>Institute of Pharmacy, University of Regensburg, Regensburg, Germany

<sup>3</sup>Center for Liver Cell Research, Department of Surgery, University Hospital Regensburg, Regensburg, Germany

<sup>4</sup>Nateco, Wolnzach; Germany

Xanthohumol (XN) is a major prenylated chalcone found in hops, which is used to add bitterness and flavor to beer. In this study, we first investigated the effects of XN on hepatocytes and hepatic stellate cells (HSC), the central mediators of liver fibrogenesis. XN inhibited the activation of primary human HSC and induced apoptosis in activated HSC *in vitro* in a dose dependent manner (0–20  $\mu$ M). In contrast, XN doses as high as 50  $\mu$ M did not impair viability of primary human hepatocytes. However, in both cell types XN inhibited activation of the transcription factor NF $\kappa$ B and expression of NF $\kappa$ B dependent proinflammatory genes. *In vivo*, feeding of XN reduced hepatic inflammation and expression of profibrogenic genes in a murine model of non-alcoholic steatohepatitis. These data indicate that XN has the potential as functional nutrient for the prevention or treatment of non-alcoholic steatohepatitis or other chronic liver disease.

Received: July 3, 2009  
Revised: August 12, 2009  
Accepted: August 24, 2009

**Keywords:**

Chalcon / Fibrosis / NASH / Steatosis / Xanthohumol

## 1 Introduction

Hops (*Humulus lupulus* L.) are included in brewing materials to add a bitter taste and flavor to beer. Xanthohumol (XN) is one of the main flavonoids in hop extracts. XN has been shown to have several biological activities, but most extensively studied is its anti-tumorigenic effect in different types of cancer. Thus, XN inhibits tumor growth and angiogenesis and induces apoptosis of tumor cells *in vitro* and *in vivo* [1–6]. More recently, amelioration of metabolic disorders by XN has been reported [7–10]. XN has been shown to decrease adipogenesis and to ameliorate lipid and

glucose metabolism in a murine model of hyperlipidaemia, obesity and type 2 diabetes [7, 10].

Obesity and insulin resistance have reached epidemic proportions worldwide, and as one of the consequences nonalcoholic fatty liver disease has emerged as a considerable public health concern. Previously, nonalcoholic fatty liver disease was often considered a relatively benign condition, but today it is evident that a significant number of patients will progress to more severe stages of liver disease including non-alcoholic steatohepatitis (NASH). In addition to fatty infiltration of the liver, NASH is characterized by inflammation, hepatocellular damage and fibrosis [11, 12].

Current evidence indicates that hepatic stellate cells (HSC) are central mediators of hepatic fibrosis in chronic liver disease including NASH. Hepatic injury results in HSC activation leading to increased proliferation and profibrogenic gene expression. Further, HSC activation is characterized by increased proinflammatory gene expression and resistance to apoptosis [13, 14]. We and others have shown that activation of the transcription factor NF $\kappa$ B plays a critical role in HSC activation [15–17]. Further, increased

**Correspondence:** Dr. Claus Hellerbrand, Department of Internal Medicine I, University Hospital Regensburg, D-93042 Regensburg, Germany

**Fax:** +49-941-944-7154

**E-mail:** claus.hellerbrand@klinik.uni-regensburg.de

**Abbreviations:** HSC, hepatic stellate cells; NASH, non-alcoholic steatohepatitis; PHH, primary human hepatocytes; TGF- $\beta$ , tumor growth factor- $\beta$ ; TNF, tumor necrosis factor; XN, xanthohumol

hepatic NF $\kappa$ B activity promotes hepatic inflammation and fibrosis in chronic liver disease including NASH [18–20]. Noteworthy, XN has been shown to exhibit its anti-inflammatory and chemopreventive effects in part *via* decreasing NF $\kappa$ B activity [2, 3]; however, its biologic activity related to fibrosis has not yet been examined.

Here, we studied the effect of XN on primary human HSC and hepatocytes *in vitro*. Further, we tested the effects of XN on hepatic inflammation and fibrogenesis in a murine NASH model.

## 2 Materials and methods

### 2.1 Cell isolation and cell culture

Isolation and culture of primary human hepatocytes (PHH) and HSC were performed as described previously [21–23]. *In vitro* activation of HSC was achieved by cell culture on uncoated tissue culture dishes as described [21, 24].

Human liver tissue for cell isolation was obtained according to the guidelines of the charitable state controlled foundation HTCR, with the informed patient's consent.

### 2.2 Chemicals

XN was obtained from Alexis Biochemicals (Lausen, Switzerland). Further, XN rich hop extract that contains XN at 73% w/w was provided by Nateco (Wolnzach, Germany). For *in vitro* experiments XN was dissolved in DMSO and added to cell culture at the indicated concentrations. Samples indicated as controls received DMSO at the same concentration as used as solvent for XN.

Tumor necrosis factor (TNF) was obtained from R&D (Wiesbaden-Nordenstadt, Germany), and Palmitic acid (Cat. No P 0500), BSA and all other chemicals from Sigma pharmaceuticals (Hamburg, Germany). Preparation of the palmitate stock solution was carried out as described previously [25]. For palmitate stimulation PHH were grown in DMEM supplemented with 0.2% FCS and palmitate at concentrations of 0.4  $\mu$ M for 24 h. 0.4% w/v FFA-free-BSA-treated cells served as controls.

### 2.3 Animals and treatment

Female BALB/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany) at 6 wk of age and housed in a 22°C controlled room under a 12-h light–dark cycle with free access to food and water. After acclimatization mice were divided into three groups (5–6 mice per group) and fed either with control diet or a NASH inducing diet [26] with or without supplementation with 1% w/w XN for 3 wks. The dose of XN was chosen based on previous *in vivo* studies in rats and mice [27–30]. The NASH diet used is also

known as Paigen diet, since it was originally developed by the group from Dr. Beverly Paigen to induce atherogenic lesions after long term feeding [31, 32]. The Paigen diet was prepared according to Matsuzawa *et al.* who recently found that feeding this diet that consists of a standard chow enriched with 15% fat (Cacao butter), cholesterol (1.25%) and cholate (0.5%) induces significant hepatic inflammation and fibrosis [26]. All three chows were prepared by Ssniff (Soest, Germany).

### 2.4 Histological analysis and measurement of hepatic cholesterol content

For histological analysis tissue specimens were fixed in 10% formalin and embedded in paraffin. Subsequently, 5- $\mu$ m sections were mounted on glass slides and stained with hematoxylin-eosin.

Hepatic cholesterol levels were measured using the cholesterol/cholesteryl ester quantification kit (BioVision, Mountain View, CA, USA) according to the manufacturer's instructions.

### 2.5 Analysis of apoptosis

For detection of apoptosis, cells were stained simultaneously with FITC-conjugated Annexin V and propidium iodide (both from Pharmingen, Germany) and analyzed by flow cytometry as described [33].

Further, the Apo-One Homogeneous Caspase-3/7 Assay (Promega, Madison, WI, USA) was used to analyze caspase-3 activity.

### 2.6 Quantification of activated nuclear NF- $\kappa$ B concentration

NF $\kappa$ B was quantified in nuclear extracts with the ELISA-based kit TransAm from Active Motif (Rixensart, Belgium) according to the manufacturer's instructions as described [34].

### 2.7 Expression analysis

Isolation of total cellular RNA from cultured cells and tissues and reverse transcription were performed as described [21]. Quantitative real-time PCR was performed with specific sets of primers (Table 1) applying LightCycler technology (Roche, Mannheim, Germany) as described [21]. Expression of IL-1 $\alpha$ , TNF, tumor growth factor- $\beta$  (TGF- $\beta$ ), and TIMP-1 was analyzed applying the QuantiTect Primer Assay according to the manufacturer's instructions (Qiagen, Hilden, Germany). Amplification of cDNA derived from 18S rRNA and  $\beta$ -actin was used for normalization in murine and human tissue, respectively.

## 2.8 Protein analysis

Protein extraction and Western blotting applying an antibody against I $\kappa$ B- $\alpha$  (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed as described [16].

## 2.9 Statistical analysis

Values are presented as mean  $\pm$  SEM. Comparison between groups was made using the Student's unpaired *t*-test. Welch's correction was performed when required. A *p* value < 0.05 was considered statistically significant. All calculations were performed using the statistical computer

package GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

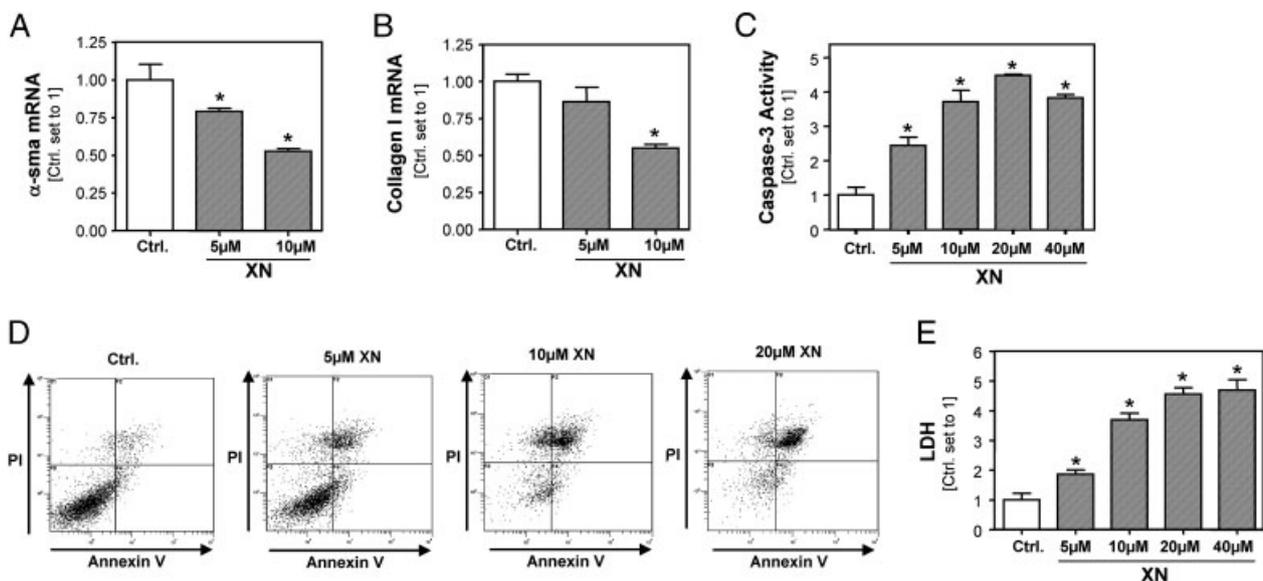
## 3 Results

### 3.1 XN inhibited the activation of HSC *in vitro*

The activation of HSC is one of the central pathophysiological mechanisms of liver fibrogenesis [35, 36]. First, we therefore aimed to analyze the effect of XN on the *in vitro* activation process of HSC. Two days after isolation human HSC were exposed to XN at two different doses (5 and 10  $\mu$ M) for 3 days. Here, and in subsequent experiments

**Table 1.** Sets of primers used for quantitative PCR analysis

Gene	Forward primer	Reverse primer
$\beta$ -Actin (human)	5'-CTA CGT CGC CCT GGA CTT CGA GC	5'-GAT GGA GCC GCC GAT CCA CAC GG
18S (murine)	5'-AAA CGG CTA CCA CAT CCA AG	5'-CCT CCA ATG GAT CCT CGT TA
Collagen I (human)	5'-CGG CTC CTG CTC CTC TT	5'-GGG GCA GTT CTT GGT CTC
Collagen I (murine)	5'-CGG GCA GGA CTT GGG TA	5'-CGG AAT CTG AAT GGT CTG ACT
$\alpha$ -sma (human)	5'-CGT GGC TAT TCC TTC GTT AC	5'-TGC CAG CAG ACT CCA TCC
IL-8 (human)	5'-TCT GCA GCT CTG TGT GAA GGT GCA GTT	5'-AAC CCT CTG CAC CCA GTT TTC CT
MCP-1 (human)	5'-CGC GAG CTA TAG AAG AAT CAC	5'-TTG GGT TGT GGA GTG AGT GT
MCP-1 (murine)	5'-TGG GCC TGC TGT TCA CA	5'-TCC GAT CCA GGT TTT TAA TGT A



**Figure 1.** Effect of xanthohumol on hepatic stellate cell activation and apoptosis *in vitro*. Two days after isolation HSC were incubated with xanthohumol (XN; 5 and 10  $\mu$ M) for 72 h. Subsequently, (A)  $\alpha$ -sma and (B) collagen I mRNA expression were analyzed by quantitative PCR analysis. *In vitro* activated HSC were incubated with different doses of XN. (C) Caspase-3 like protease activity was measured in cytosolic protein extracts by cleavage of the fluorogenic substrate Z-DEVD-Rhodamine-110. Activities are represented as fold-increase of Rhodamine-110 fluorescence over control. (D) Apoptosis was analyzed by flow cytometry applying Annexin V and propidium iodid staining. Annexin V<sup>+</sup> and PI cells reflect early apoptosis while Annexin V<sup>+</sup> and PI<sup>+</sup> cells indicate late apoptosis/secondary necrosis. (E) Twenty-four hours after XN stimulation vitality was measured as cell membrane lysis and release of lactate dehydrogenase (LDH) into supernatants. (\**p* < 0.05 compared to control).

control cells were treated with DMSO at the same concentration as used as solvent for XN. Subsequently, mRNA expression of two established markers of HSC activation, namely collagen type I and alpha-smooth muscle actin ( $\alpha$ -sma), was determined by quantitative RT-PCR analysis. Treatment with XN significantly reduced the expression of collagen type I (Fig. 1A) and  $\alpha$ -sma (Fig. 1B) compared to control HSC.

### 3.2 XN-induced apoptosis in activated HSC *in vitro*

Once they are activated HSC are characterized by high resistance to apoptosis, a mechanism that has therefore been proposed to play a key role in the progression of fibrosis in chronic liver disease. Incubation of *in vitro* activated HSC with XN for 6 h led to dose-dependent (0–20  $\mu$ M) activation of caspase-3 (Fig. 1C). Incubation of HSC with higher doses of XN led to detachment of HSC (data not shown). After 24 h incubation with 10  $\mu$ M or 20  $\mu$ M XN, almost all cells appeared positive for propidium iodid, indicating late apoptosis and necrosis (Fig. 1D). In line with these data, secretion of LDH dose-dependently increased 24 h after XN treatment and reached a plateau at 20  $\mu$ M (Fig. 1E).

### 3.3 XN inhibited NF $\kappa$ B activity and proinflammatory gene expression of activated HSC *in vitro*

XN is known to inhibit NF $\kappa$ B activity in tumorous cells [3], and we and others have shown that NF $\kappa$ B activity is crucial for both HSC activation and resistance to apoptosis [15, 17]. Here, we found that XN reduced both basal as well as TNF induced NF $\kappa$ B activity in nuclear extracts of activated HSC (Fig. 2A). Furthermore, XN repressed TNF mediated I $\kappa$ B- $\alpha$  degradation in activated HSC (Fig. 2B). In accordance, XN

impaired TNF-induced MCP-1 expression, a proinflammatory chemokine that is *de novo* expressed during HSC activation and that is highly regulated via activation of the transcription factor NF $\kappa$ B in activated HSC [16] (Fig. 2C).

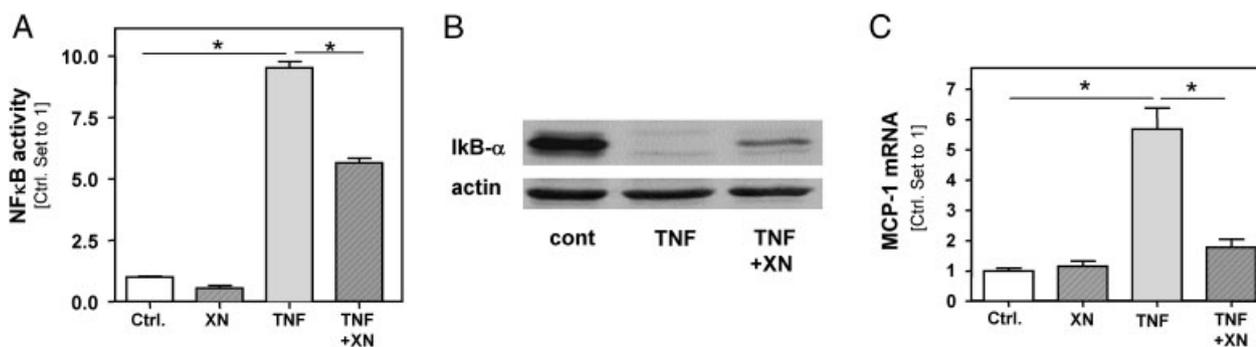
### 3.4 XN did not impair viability but inhibited proinflammatory gene expression of hepatocytes

*In vitro* effects on HSC were achieved at the same or even lower concentrations as observed in human cancer cells of different origin [2, 4–6]. However, data regarding apoptotic or cytotoxic effects on PHH were missing so far.

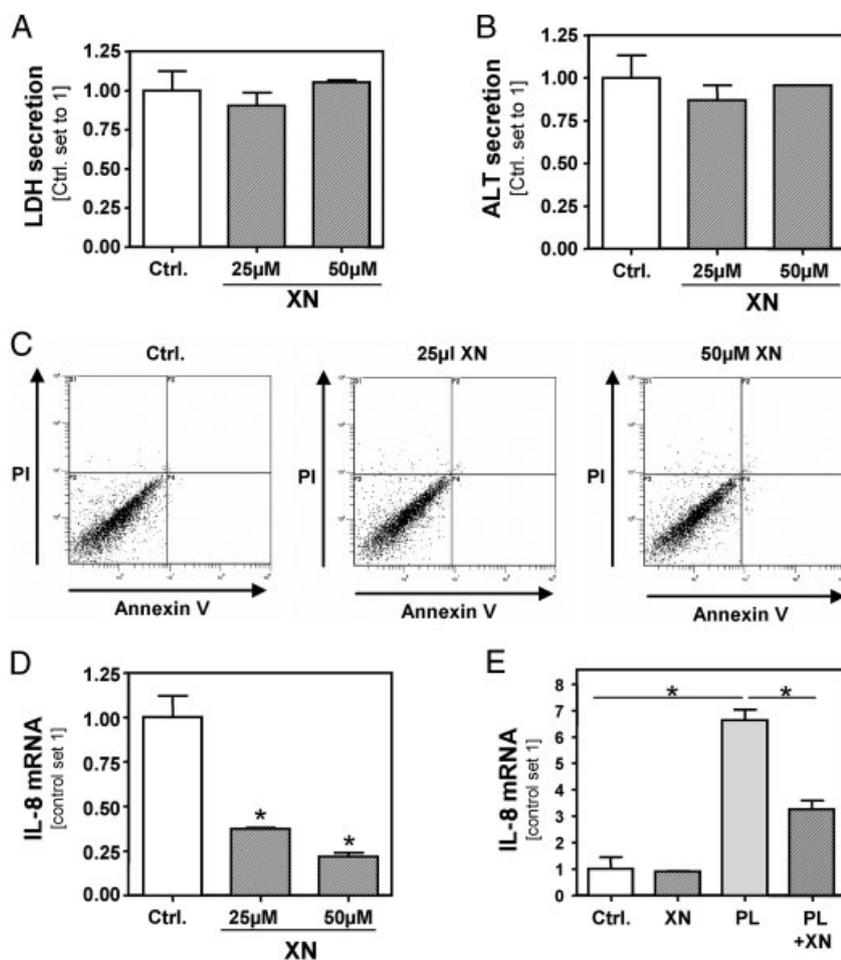
Noteworthy, XN did not affect LDH (Fig. 3A) or ALT (Fig. 3B) levels in the supernatant of PHH incubated with XN doses as high as 50  $\mu$ M for 24 h. FACS analysis confirmed that there was no significant apoptosis or necrosis in PHH after 24-h stimulation with 25 or 50  $\mu$ M XN (Fig. 3C). However, at the same concentrations a significant inhibition of IL-8 expression, another chemokine known to be regulated by NF $\kappa$ B, was observed (Fig. 3D). Recent studies have shown that free fatty acids are capable of inducing NF $\kappa$ B and proinflammatory gene expression in hepatocytes [37]. Here, we confirmed significant induction of IL-8 expression in PHH following stimulation with 0.4  $\mu$ M palmitate, and this induction was inhibited by simultaneous incubation with XN (Fig. 3E).

### 3.5 XN did not affect hepatic steatosis in a murine NASH model

*In vitro* data indicate that XN exhibits antifibrogenic effects at concentrations that do not affect the viability of PHH but



**Figure 2.** Effect of xanthohumol on NF $\kappa$ B activity and proinflammatory gene expression of activated HSC *in vitro*. After 24 h serum depletion activated human HSC were stimulated with xanthohumol (XN; 5  $\mu$ M), and subsequently, with TNF (10 ng/mL) in serum-free medium. (A) NF- $\kappa$ B activity in nuclear extracts of TNF stimulated (2 h) and control cells. (B) Analysis of I $\kappa$ B- $\alpha$  in protein extracts of TNF stimulated (30 min) and control cells by Western blotting. (C) MCP-1 mRNA expression in TNF stimulated (24 h) and control cells analyzed by qPCR. (\* $p$  < 0.05 compared to control.)



**Figure 3.** Effect of xanthohumol on viability and proinflammatory gene expression of hepatocytes *in vitro*. Primary human hepatocytes (PHH) were incubated with XN for 24 h at the concentrations indicated. Vitality was assessed as release of LDH (A) and ALT (B) into the supernatants (C) Analysis of apoptosis by FACS-analysis using Annexin V/PI-staining. (D) Analysis of IL-8 mRNA expression by quantitative PCR in XN (20 µM) stimulated and control PHH. (E) IL-8 mRNA expression in PHH stimulated with palmitate (PL; 0.4 µM) or palmitate and XN (20 µM) for 24 h. PHH stimulated with the solvents DMSO and BSA served as control. (\* $p < 0.05$  compared to control.)

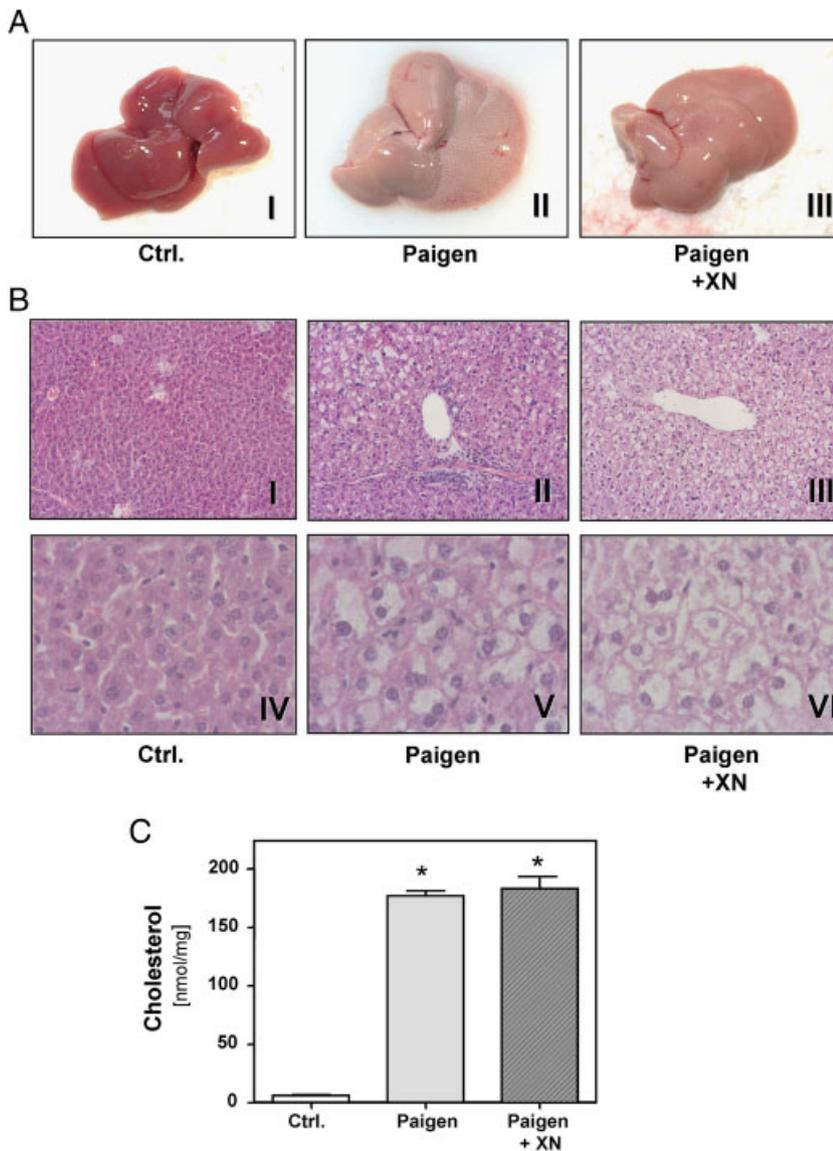
even suppress basal as well as free fatty acid induced expression of proinflammatory chemokines known to play a role in progression of NASH [6, 38, 39]. These *in vitro* findings encouraged us to test the effect of XN in a dietary NASH model, named Paigen diet [31, 32], in mice. We selected this model since recently it has been described that feeding this diet induced significant hepatic inflammation and fibrogenesis already after 6 or 24 wk, respectively [26]. Here, we applied the Paigen diet either alone or supplemented with 1% XN w/w for 3 wk (due to limited availability of XN). Mice receiving standard chow served as control.

No significant differences were found between treatment groups regarding food and fluid intake or body weight throughout the study (data not shown). Despite the short feeding period, the Paigen-diet induced macroscopically visible hepatic steatosis that appeared similar in the Paigen+XN group (Fig. 4A). Histological analysis revealed microvesicular steatosis in both mice fed the Paigen-diet alone or in combination with XN (Fig. 4B). It had been shown that cholesterol is the predominant lipid accumulating in the liver after Paigen-feeding.

Also here, we found a significant increase of hepatic cholesterol levels after 3 wk feeding this diet, and cholesterol levels did not differ between the Paigen and the Paigen+XN group (Fig. 4C).

### 3.6 XN inhibited hepatic inflammation in a murine NASH model

In addition to steatosis, histological analysis revealed significant inflammation and necrosis in mice fed with the Paigen-diet (Fig. 4B, II), but these histopathological changes were apparently less pronounced in the Paigen+XN group (Fig. 4B, III). In accordance, ALT (Fig. 5A) and AST (data not shown) serum levels were significantly increased in the Paigen-group but reduced to normal levels in mice fed Paigen+XN. Further, both TNF and IL-1 expression were significantly increased in mice fed the Paigen-diet (Fig. 5B and C), but this increase was almost completely blunted in mice fed Paigen+XN. Similarly, MCP-1 mRNA was significantly increased in mice fed the PL Paigen-diet compared to control fed mice,



**Figure 4.** Effect of xanthohumol on hepatic steatosis in a murine NASH model. Mice were fed with the NASH inducing Paigen-diet either alone or supplemented with xanthohumol (Paigen+XN) for 3 wk. Mice receiving standard chow served as control. (A) Representative macroscopic images of the livers of the three treatment groups. (B) HE-staining of liver tissue from mice fed Paigen (II, V), Paigen+XN (III, VI) or control mice (I, IV). Representative images at two different magnifications are shown. (C) Intrahepatic cholesterol levels. (\* $p < 0.05$  compared to control.)

but the increase was diminished in the Paigen+XN group (Fig. 5D).

### 3.7 XN inhibited profibrogenic gene expression in a murine NASH model

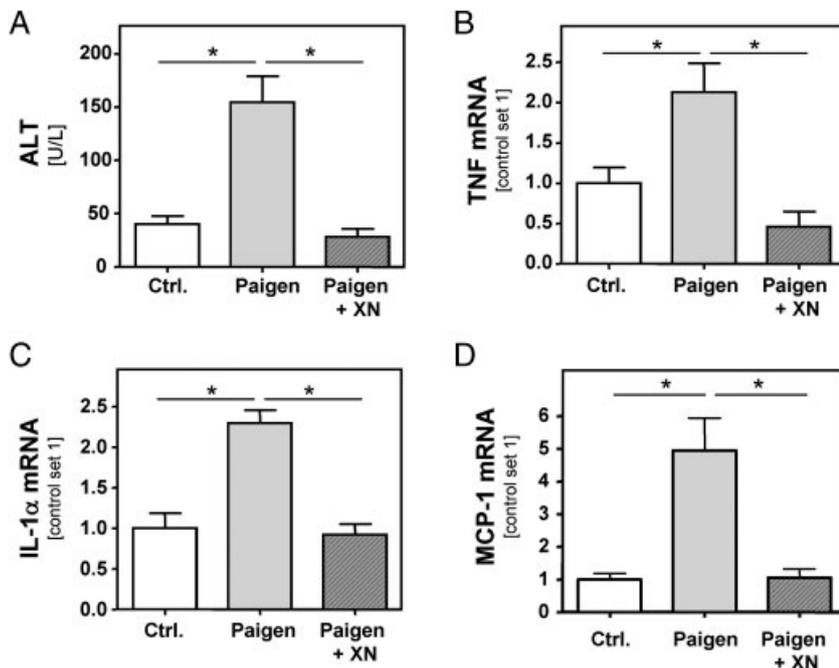
Besides inflammatory gene expression, a significant increase of the mRNA levels of the profibrogenic genes TGF- $\beta$  and TIMP-1 was observed in mice fed the Paigen-diet (Fig. 6A and B). In contrast, hepatic mRNA levels of both profibrogenic genes in Paigen-XN mice did not differ significantly from control mice. After 3 wk feeding the Paigen-diet we did not yet observe hepatic fibrosis in histological analysis; however, collagen type I mRNA was significantly increased in Paigen-diet but not in

Paigen+XN fed mice compared to mice fed control diet (Fig. 6C).

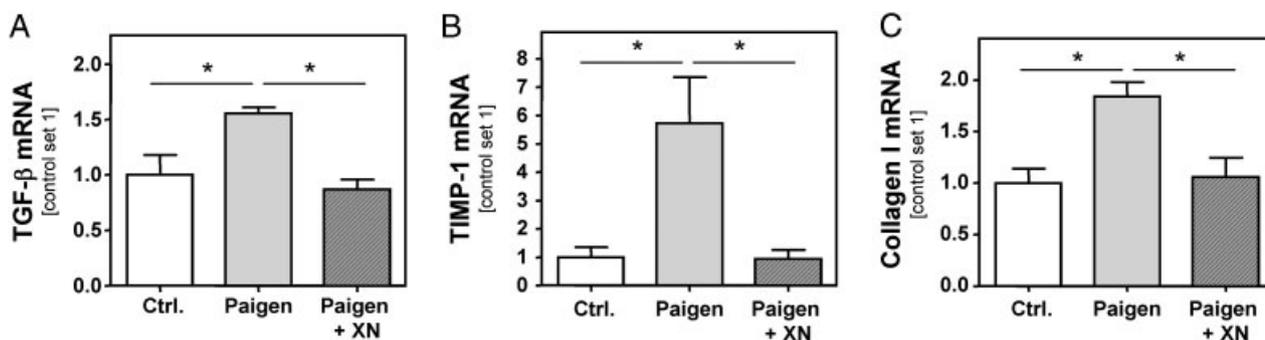
## 4 Discussion

In the present study we aimed to analyze the effects of XN on liver cells and its biological activity in a murine model of chronic liver disease.

It is noteworthy that XN affected different pathophysiological mechanisms relevant for liver fibrosis *in vitro*. HSC activation is inhibited while apoptosis of activated HSC is induced, respectively, at XN concentrations as low as 5  $\mu\text{M}$ . In contrast and importantly, tenfold higher XN concentrations (50  $\mu\text{M}$ ) did not induce cytotoxic effects in PHH *in vitro*. Moreover, XN inhibited MCP-1 and IL-8 expression in



**Figure 5.** Effect of xanthohumol on hepatic inflammation in a murine NASH model. Mice were fed the Paigen-diet either alone or supplemented with xanthohumol (Paigen+XN) for 3 wk. Mice receiving standard chow served as control. (A) ALT serum levels. Analysis of hepatic mRNA levels of (B) TNF, (C) IL-1 and (D) MCP-1 by quantitative RT-PCR. (\* $p < 0.05$ .)



**Figure 6.** Effect of xanthohumol on profibrogenic gene expression in a murine NASH model. Mice were fed the Paigen-diet either alone or supplemented with xanthohumol (Paigen+XN) for 3 wk. Mice receiving standard chow served as control. Analysis of hepatic mRNA levels of (A) TGF- $\beta$  (B) TIMP-1 and (C) Collagen I by quantitative RT-PCR. (\* $p < 0.05$ .)

HSC as well as PHH. Both chemokines are regulated by NF $\kappa$ B and increased levels are associated with fibrosis progression in NASH [20]. Further, NF $\kappa$ B activation is a central pathophysiological mechanism during HSC activation [15], and importantly, XN inhibited basal as well as cytokine induced NF $\kappa$ B activity in HSC *in vitro*.

Based on these *in vitro* findings and the recently reported potential of XN to ameliorate metabolic disorders [7], we decided to apply an experimental NASH model to test the effect of XN on hepatic inflammation and fibrogenesis *in vivo*. We applied a dietary model that has been recently shown to resemble the pathology of human NASH including HSC activation [26].

It is known that steatosis is mainly caused by cholesterol in this model and that cholesterol induced oxidative stress is responsible for hepatic inflammation and NF $\kappa$ B activation

[26, 40]. In line with this, we found a significant increase of hepatic cholesterol levels in mice fed this diet but this increase was not affected by the addition of XN to the NASH inducing diet. However and strikingly, despite the fact that steatosis has not been affected by XN in this model, hepatic inflammation, and profibrogenic gene expression were almost completely blunted in mice fed XN. Thus, one may hypothesize that XN has therapeutic efficacy also in liver injury not related to steatosis.

Little is known about the metabolism and bioavailability of XN. It has been shown that XN is effectively metabolized by rat and human microsomes *in vitro* [41] suggesting that XN is probably completely metabolized in the liver *in vivo*. However, detailed information regarding the bioavailability or the gastrointestinal uptake rate following oral administration is elusive. Here, we used XN dose

(1 mg/g BW/day) in the same range as in previous *in vivo* studies [27–30].

For humans, beer is the major dietary source of XN, but probably, in general, the average content of XN in beer is not high enough to produce a protective effect. However, XN levels vary significantly depending on the type of beer. Lager and pilsener beers have fairly low levels of this compound, and highest levels are found in stout or porter [42]. Further, a brewing process has been developed that produces a beer that contains ten times the amount of XN as traditional brews [43]. Nevertheless, there is unanimous hesitancy regarding XN uptake *via* beer because of the fine line between moderate and binge drinking. However, XN can also be isolated from hops in large quantities, and thus, independent of beer intake XN may be used as a dietary supplement for the prevention or treatment of NASH or other chronic liver disease. Still, further safety and efficacy studies are required and more research is needed before it is known whether the findings of the present study can be applied to humans. Thus, recent reports indicate that XN has positive effects on lipid and glucose metabolism [7, 10], however, it also has been shown that XN may contribute to hypertrophy of adipocytes [44]. Therefore, future studies may focus on XN effects on (visceral) fat in addition to hepatic tissue. Further, XN has been shown to affect aromatase activity, and herewith, estrogen formation, which may be considered particularly upon long term application [45]. However, currently available animal toxicity and safety studies [27, 28] and our *in vitro* experiments using human hepatocytes provide evidence that XN may not be harmful to humans. Therefore, it is promising that the present study newly revealed the potential of XN as a functional nutrient to inhibit inflammation and fibrogenesis in chronic liver disease.

*The authors thank Monika Artinger for excellent technical assistance. This work was supported by grants from the German Research Association (He 2458/14-1 and Schn 620/3-1 to C.H.) and the Medical Faculty of the University of Regensburg (ReForM) to T.S.W. and C.H. Further, this project was supported in part by an unrestricted research grant from the Joh. Barth & Sohn GmbH (Nuremberg, Germany). Financial relationships of the authors with Joh. Barth & Sohn GmbH are as follows: C.H. is a consultant, and C.D. is working in the laboratory of C.H.M.G. is an employee of NATECO2 a members of the Barth-Haas Group. All other authors declare that they do not have a financial relationship with Joh. Barth & Sohn GmbH or another company related to this project. All authors had complete and independent control over the study design, analysis and interpretation of data, report writing, and publication, regardless of results.*

*The authors have declared no conflict of interest.*

## 5 References

- [1] Monteiro, R., Calhau, C., Silva, A. O., Pinheiro-Silva, S. *et al.*, Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J. Cell Biochem.* 2008, 104, 1699–1707.
- [2] Dell'Eva, R., Ambrosini, C., Vannini, N., Piaggio, G. *et al.*, AKT/NF-kappaB inhibitor xanthohumol targets cell growth and angiogenesis in hematologic malignancies. *Cancer* 2007, 110, 2007–2011.
- [3] Albin, A., Dell'Eva, R., Vene, R., Ferrari, N. *et al.*, Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF-kappaB and Akt as targets. *FASEB J.* 2006, 20, 527–529.
- [4] Delmulle, L., Bellahcene, A., Dhooge, W., Comhaire, F. *et al.*, Anti-proliferative properties of prenylated flavonoids from hops (*Humulus lupulus* L.) in human prostate cancer cell lines. *Phytomedicine* 2006, 13, 732–734.
- [5] Colgate, E. C., Miranda, C. L., Stevens, J. F., Bray, T. M., 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.
- [6] Miranda, C. L., Stevens, J. F., Helmrich, A., Henderson, M. C. *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.
- [7] 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.
- [8] Casaschi, A., Maiyoh, G. K., Rubio, B. K., Li, R. W. *et al.*, The chalcone xanthohumol inhibits triglyceride and apolipoprotein B secretion in HepG2 cells. *J. Nutr.* 2004, 134, 1340–1346.
- [9] Tabata, N., Ito, M., Tomoda, H., Omura, S., Xanthohumols, diacylglycerol acyltransferase inhibitors, from *Humulus lupulus*. *Phytochemistry* 1997, 46, 683–687.
- [10] Yang, J. Y., Della-Fera, M. A., Rayalam, S., Baile, C. A., Effect of xanthohumol and isoxanthohumol on 3T3-L1 cell apoptosis and adipogenesis. *Apoptosis* 2007, 12, 1953–1963.
- [11] Powell, E. E., Cooksley, W. G., Hanson, R., Searle, J. *et al.*, The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990, 11, 74–80.
- [12] Caldwell, S. H., Oelsner, D. H., Iezzoni, J. C., Hespender, E. E. *et al.*, Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999, 29, 664–669.
- [13] Bataller, R., Brenner, D. A., Liver fibrosis. *J. Clin. Invest.* 2005, 115, 209–218.
- [14] Friedman, S. L., Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. *Nat. Clin. Pract. Gastroenterol. Hepatol.* 2004, 1, 98–105.
- [15] Elsharkawy, A. M., Oakley, F., Mann, D. A., The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis* 2005, 10, 927–939.
- [16] Hellerbrand, C., Jobin, C., Licato, L. L., Sartor, R. B., Brenner, D. A., Cytokines induce NF-kappaB in activated but not in quiescent rat hepatic stellate cells. *Am. J. Physiol.* 1998, 275, G269–G278.

- [17] Hellerbrand, C., Jobin, C., Imuro, Y., Licato, L. *et al.*, Inhibition of NF- $\kappa$ B in activated rat hepatic stellate cells by proteasome inhibitors and an I $\kappa$ B super-repressor. *Hepatology* 1998, 27, 1285–1295.
- [18] Elsharkawy, A. M., Mann, D. A., Nuclear factor- $\kappa$ B and the hepatic inflammation-fibrosis-cancer axis. *Hepatology* 2007, 46, 590–597.
- [19] Beraza, N., Malato, Y., Vander, B. S., Liedtke, C. *et al.*, Pharmacological IKK2 inhibition blocks liver steatosis and initiation of non-alcoholic steatohepatitis. *Gut* 2008, 57, 655–663.
- [20] Marra, F., Nuclear factor- $\kappa$ B inhibition and non-alcoholic steatohepatitis: inflammation as a target for therapy. *Gut* 2008, 57, 570–572.
- [21] Muhlbauer, M., Bosserhoff, A. K., Hartmann, A., Thasler, W. E. *et al.*, A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. *Gastroenterology* 2003, 125, 1085–1093.
- [22] Hellerbrand, C., Amann, T., Schlegel, J., Wild, P. *et al.*, The novel gene MIA2 acts as a tumour suppressor in hepatocellular carcinoma. *Gut* 2008, 57, 243–251.
- [23] Hellerbrand, C., Bumès, E., Bataille, F., Dietmaier, W. *et al.*, Reduced expression of CYLD in human colon and hepatocellular carcinomas. *Carcinogenesis* 2007, 28, 21–27.
- [24] Muhlbauer, M., Fleck, M., Schutz, C., Weiss, T. *et al.*, PD-L1 is induced in hepatocytes by viral infection and by interferon- $\alpha$  and - $\gamma$  and mediates T cell apoptosis. *J. Hepatol.* 2006, 45, 520–528.
- [25] Cousin, S. P., Hugl, S. R., Wrede, C. E., Kajio, H. *et al.*, Free fatty acid-induced inhibition of glucose and insulin-like growth factor I-induced deoxyribonucleic acid synthesis in the pancreatic beta-cell line INS-1. *Endocrinology* 2001, 142, 229–240.
- [26] Matsuzawa, N., Takamura, T., Kurita, S., Misu, H. *et al.*, Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. *Hepatology* 2007, 46, 1392–1403.
- [27] Vanhoecke, B. W., Delporte, F., Van Braeckel, E., Heyerick, A. *et al.*, A safety study of oral tangeretin and xanthohumol administration to laboratory mice. *In Vivo* 2005, 19, 103–107.
- [28] Hussong, R., Frank, N., Knauft, J., Ittrich, C. *et al.*, A safety study of oral xanthohumol administration and its influence on fertility in Sprague Dawley rats. *Mol. Nutr. Food Res.* 2005, 49, 861–867.
- [29] Nookandeh, A., Frank, N., Steiner, F., Ellinger, R. *et al.*, Xanthohumol metabolites in faeces of rats. *Phytochemistry* 2004, 65, 561–570.
- [30] Avula, B., Ganzera, M., Warnick, J. E., Feltenstein, M. W. *et al.*, High-performance liquid chromatographic determination of xanthohumol in rat plasma, urine, and fecal samples. *J. Chromatogr. Sci.* 2004, 42, 378–382.
- [31] Nishina, P. M., Verstuyft, J., Paigen, B., Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J. Lipid Res.* 1990, 31, 859–869.
- [32] Nishina, P. M., Lowe, S., Verstuyft, J., Naggert, J. K. *et al.*, Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J. Lipid Res.* 1993, 34, 1413–1422.
- [33] Muhlbauer, M., Fleck, M., Schutz, C., Weiss, T. *et al.*, PD-L1 is induced in hepatocytes by viral infection and by interferon- $\alpha$  and - $\gamma$  and mediates T cell apoptosis. *J. Hepatol.* 2006, 45, 520–528.
- [34] Muhlbauer, M., Allard, B., Bosserhoff, A. K., Kiessling, S. *et al.*, Differential effects of deoxycholic acid and taurodeoxycholic acid on NF- $\kappa$ B signal transduction and IL-8 gene expression in colonic epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2004, 286, G1000–G1008.
- [35] Bataller, R., Brenner, D. A., Liver fibrosis. *J. Clin. Invest.* 2005, 115, 209–218.
- [36] Friedman, S. L., Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008, 134, 1655–1669.
- [37] Joshi-Barve, S., Barve, S. S., Amancherla, K., Gobejishvili, L. *et al.*, Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. *Hepatology* 2007, 46, 823–830.
- [38] Haukeland, J. W., Damas, J. K., Konopski, Z., Loberg, E. M. *et al.*, Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J. Hepatol.* 2006, 44, 1167–1174.
- [39] Jarrar, M. H., Baranova, A., Collantes, R., Ranard, B. *et al.*, Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* 2008, 27, 412–421.
- [40] Wouters, K., van Gorp, P. J., Bieghs, V., Gijbels, M. J. *et al.*, Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* 2008, 48, 474–486.
- [41] Yilmazer, M., Stevens, J. F., Buhler, D. R., *In vitro* glucuronidation of xanthohumol, a flavonoid in hop and beer, by rat and human liver microsomes. *FEBS Lett.* 2001, 491, 252–256.
- [42] Stevens, J. F., Taylor, A. W., Deinzer, M. L., Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* 1999, 832, 97–107.
- [43] Wunderlich, S., Zurcher, A., Back, W., Enrichment of xanthohumol in the brewing process. *Mol. Nutr. Food Res.* 2005, 49, 874–881.
- [44] 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.
- [45] Monteiro, R., Faria, A., Azevedo, I., Calhau, C., Modulation of breast cancer cell survival by aromatase inhibiting hop (*Humulus lupulus* L.) flavonoids. *J. Steroid Biochem. Mol. Biol.* 2007, 105, 124–130.