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Xanthohumol feeding does not impair organ function and homoeostasis in mice

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ABSTRACT

Xanthohumol, the major prenylated chalcone found in hops, is known to exert several beneficial effects but only few studies evaluated the safety profile of this natural compound with in part discrepant results. Here, we fed female BALB/c mice with a standard diet supplemented with xanthohumol for 3 weeks, and thus, achieved a daily dose of approximately 1000 mg xanthohumol/kg body weight. There were no significant differences in body weight or food intake between mice on standard diet and animals receiving the same diet supplemented with xanthohumol. Histopathological examination of liver, kidney, colon, lung, heart, spleen and thymus revealed no signs of xanthohumol-toxicity, and biochemical serum analysis confirmed normal organ function. Further, xanthohumol treatment did not affect hepatic glycogen content CYP2E1 and CYP1A2 expression levels, but CYP3A11 mRNA was approximately 30% reduced. Expression of several genes indicative of early hepatic inflammation and fibrosis, a hallmark of chronic liver injury, did not differ between xanthohumol treated and control mice. In summary, these results indicate that oral administration of xanthohumol exhibits no adverse effects on major organ function and homoeostasis in mice. Particularly, hepatotoxic effects could be ruled out confirming a good safety profile of xanthohumol as prerequisite for further studies in humans.

1. Introduction

Hops, the female inflorescences of the hop plant *Humulus lupulus* L. have been used for more than 500 years to preserve beer and to give it a characteristic aroma and flavor (Neve, 1991). However, hop is known even longer as a medical plant to treat a variety of complaints such as sleeplessness and nervousness (Blumenthal, 1998), anorexia and indigestions (Barnes et al., 2002; Grieve, 1971), as well as headache, rheumatism, bladder inflammation and climacteric complaints (Blumenthal, 1998; Bown, 2001; Hamel and Chiltoskey, 1975; Zanoli and Zavatti, 2008). In line with the growing interest in health benefits of plants used in traditional medicine, *H. lupulus* has received much attention, particularly, its polyphenolic compounds.

Especially for xanthohumol (XN), the principle prenylchalcone of hops, a wide range of biological effects were reported. XN exhibits anti-inflammatory (Albini et al., 2006; Cho et al., 2008; Colgate et al., 2007; Gerhauser et al., 2002), anti-oxidant (Gerhauser et al., 2002; Vogel and Heilmann, 2008; Vogel et al., 2008; Yamaguchi et al., 2009) and anti-angiogenic (Albini et al., 2006; Bertl et al., 2004, 2006) effects. Further, XN exhibits anti-infective activity against bacteria (Bhattacharya et al., 2003; Gerhauser, 2005b; Mizobuchi and Sato, 1984), viruses (Buckwold et al., 2004; Gerhauser, 2005b; Wang et al., 2004), fungi (Gerhauser, 2005b; Mizobuchi and Sato, 1984) and plasmodia (Frolich et al., 2005; Gerhauser, 2005b; Herath et al., 2003; Li et al., 1995, 2001). Most extensively studied is its anti-tumorigenic effect on different types of cancer cells including colon, ovarian, breast and colon cancer or fibrosarcoma (Colgate et al., 2007; Delmulle et al., 2006; Goto et al., 2005; Miranda et al., 1999; Pan et al., 2005; Vanhoecke et al., 2005a).

In an *in vivo* study in mice, oral administration of approximately 13 mg XN/kg body weight (b.w.) via drinking water showed anticancerous effects (Albini et al., 2006). Still, little is known about the bioavailability and the metabolism of XN particularly after oral administration. Previous studies in rats indicate that the bioavailability of XN after oral administration is very low (Avula et al., 2004; Nookandeh et al., 2004). Further, these studies indicated a maximal tolerable dose of more than 1000 mg XN/kg b.w. since no adverse effects were observed in rats after single oral application of this dose. So far, two long term safety studies of XN have been reported. Vanhoecke et al. applied a daily dose of approximately 35 mg XN/kg b.w. per day to mice for 4 weeks, and did not observe any noticeable signs of toxicity (Vanhoecke et al., 2005b). Particularly, there were no signs of hepatotoxicity and

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differences regarding lipid or carbohydrate metabolism. In a second study, Hussong et al. applied 1000 mg XN/kg b.w. per day by gavage to female Sprague Dawley rats for 4 weeks (Hussong et al., 2005). Also in this carefully performed study no remarkable treatment-related changes were observed in most organs, and importantly, there were no adverse effects on female reproduction or the development of offspring. However, in XN treated rats liver weight was reduced and histological investigation indicated a loss of hepatic glycogen suggestive of mild hepatoxicity. Particularly with respect to long term application even weak hepatoxicity is a critical issue since it harbors the risk to progress to chronic hepatic inflammation and fibrosis (Ramachandran and Kakar, 2009; Stravitz and Sanyal, 2003).

However, total liver weight and glycogen content are only unspecific and vague signs of hepatotoxicity. Furthermore, based on the studies of Hussong et al. (2005) and Vanhoecke et al. (2005b) it is not clear whether the different XN doses or species differences account for the differing results regarding potential hepatoxicity.

Thus, in the present study, we aimed to detect possible side effects of high dose XN (1000 mg/kg b.w. per day) chronically fed to mice with an emphasis on liver-function and -homoeostasis.

2. Methods

2.1. Reagents

Xanthohumol was obtained from Alexis Biochemicals (Lausen, Switzerland). All other chemicals were obtained from Sigma Pharmaceuticals (Hamburg, Germany).

2.2. Animals and treatment

Female BALB/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany) at 6 weeks 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 1 week of acclimatization mice were divided into 2 groups (6 mice per group) and fed either with standard diet (control) or standard diet supplemented with XN (0.5% (w/w)) for 3 weeks. Both chows were prepared by Ssniff (Soest, Germany). At the end of the experimental time, mice were killed by heart puncture under ketamine/xylazine (2:1) anesthesia, and blood samples and organs were collected for further analyses.

2.3. Histological analysis

For histological analysis tissue specimens were fixed for 24 h in buffered formaldehyde solution (3.7% in PBS) at room temperature, dehydrated by graded ethanol and embedded in paraffin. Tissue sections (thickness 5 μm) were deparaffinized with xylene and stained with eosin/haematoxylin (H&E). Digital images were captured with an Olympus CKX41 microscope equipped with the ALTRA20 Soft Imaging System (Olympus, Hamburg, Germany).

2.4. Expression analysis

Isolation of total cellular RNA from frozen liver sections, reverse transcription and quantification of mRNA expression using LightCycler real-time PCR technology (Roche) were performed as described (Muhlbauer et al., 2003) applying the following pairs of primers: collagen type I (coll-I) forward: 5′-CGG GCA GGA CTT GGG TA-3′ and coll-I reverse: 5′-CGG AAT CTG AAT GGT CTG ACT-3′; monocyte chemoattractant protein-1 (MCP-1) forward: 5′-TGG GCC TGC TGT TCA CA-3′ and MCP-1 reverse: 5′-TCC GAT CCA GGT TTT TAA TGT A-3′. Expression of CYP2E1, tumor necrosis factor (TNF), interleukin-1 *alpha* (IL-1α), intercellular adhesion molecule-1 (ICAM-1), p47phox, and transforming growth factor-*beta* (TGF-β) was analyzed applying the QuantiTect Primer Assay according to the manufacturer's instructions (Qiagen, Hilden, Germany). Amplification of cDNA derived from 18S rRNA was used for normalization (18s forward: 5′-AAA CGG CTA CCA CAT CCA AG-3′ and 18S reverse: 5′-CCT CCA ATG GAT CCT CGT TA-3′).

2.5. Glycogen assay

Hepatic glycogen content was quantified using the Glycogen Assay Kit from BioVision (Heidelberg, Germany) according to the manufacturer's instructions. Briefly, frozen liver tissue sections were weighed and homogenized in ddH_2O under cooling. Thereafter, enzymes were inactivated by boiling the homogenates for 5 min. Then, glycogen was hydrolyzed to glucose applying the provided hydrolyses enzyme mix and quantified colorimetrically using an EMax Microplate Reader (MWG Biotech, Ebersberg, Germany).

2.6. Quantification of endotoxin serum levels (LAL assay)

For quantification of endotoxin serum levels, blood was collected and processed under sterile and pyrogen-free conditions and serum was stored in endotoxin free cups. Endotoxin concentration was determined using the Limulus Amebocyte Lysate (LAL) assay from Hycult Biotechnology (Uden, The Netherlands) according to the manufacturer's instructions.

2.7. Statistical analysis

Values are presented as mean ± SEM or mean ± SD as indicated. 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. In-life parameters showed no significant difference between experimental groups

To study potential toxic effects of orally applied xanthohumol (XN) *in vivo*, we fed mice with standard chow supplemented with 0.5% (w/w) XN for 3 weeks resulting in a daily dose of approximately 1000 mg/kg b.w. per day. Control mice received the same chow without XN-supplementation. Monitoring of daily food intake (Fig. 1A, 3.2 ± 0.20 g XN supplemented chow per mouse and day) confirmed that we achieved this goal (average XN dose 998 ± 21 mg XN/kg b.w. per day), and daily food and herewith XN consumption, respectively, did not significantly change over

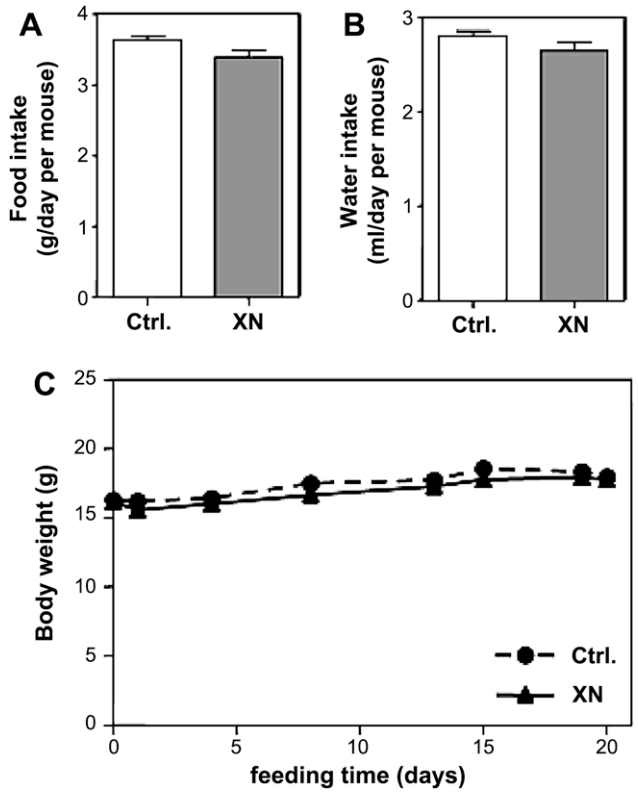

Fig. 1. Analysis of in-life parameters during oral xanthohumol feeding. Mean daily (A) food and (B) water intake (per mouse), and (C) mean total body weight during the experimental period (3 weeks) of xanthohumol fed (XN; 1000 mg XN/kg b.w. per day) and control (Ctrl.) mice.