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ORIGINAL ARTICLE

Antifibrotic effects of crocin on thioacetamide-induced liver fibrosis in mice

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Abstract Liver fibrosis is a major health concern that results in significant morbidity and mortality. Up-to-date, there is no standard treatment for fibrosis because of its complex pathogenesis. Crocin is one of the main nutraceuticals isolated from the stigma of *Crocus sativus* with antioxidant and anti-inflammatory activities. The current study aimed at evaluating the potential antifibrotic activity of crocin against thioacetamide (TAA)-induced liver fibrosis in mice as well as the underlying mechanism using silymarin as a reference antifibrotic product. Crocin at two doses (25 and 100 mg/kg) significantly ameliorated the rise in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and total bilirubin (TB). Further, the high dose significantly protected against the increase in serum total cholesterol (TC) and triglycerides (TG). These effects were confirmed by light microscopic examinations. Crocin antioxidant activities were confirmed by the observed inhibition of reduced glutathione depletion (GSH), super oxide dismutase (SOD) exhaustion and malondialdehyde (MDA) accumulation in liver tissue. The antifibrotic effects of crocin were explored by assessing fibrosis related gene expression. Administration of crocin (100 mg/kg) hampered expression of tumor growth factor-β (TGF-β), α alpha smooth muscle actin (α-SMA) and collagen 1-α expression and significantly raised gene expression of matrix metalloproteinase-2 (MMP-2). Further, it reduced protein expression of nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX-2) as assessed immunohistochemically. These anti-inflammatory effects were confirmed by the observed protein expression of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Thus, it can be concluded that crocin protects against TAA-induced liver fibrosis in mice. This can be ascribed, at least partly, to its antioxidant and anti-inflammatory effects.

1. Introduction

Liver fibrosis is a major health concern that results in significant morbidity and mortality (Sánchez-Valle et al., 2012). The pathogenesis of liver fibrosis is complex and involves deposition of extracellular matrix (ECM) by activated hepatic stellate cells (HSC) (Reeves and Friedman, 2002). Inflamma-

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tion, induced by oxidative stress, is a key event in HSC activation (Greenwel et al., 2000). Several pro-inflammatory cytokines, chemokines and adhesion molecules initiate the activation of HSC (Pinzani and Macias-Barragan, 2010). There is no FDA approved medication for liver fibrosis (Bataller and Brenner, 2005). Several attempts have been made to combat liver fibrosis by inhibiting known molecular targets or pathways that are critically involved in the pathogenesis of fibrogenesis.

Prevention or attenuation of the activation of hepatic stellate and Kupffer cells has been given much attention. Those two cells are responsible for propagation of oxidative and inflammatory responses, with subsequent activation of various fibrogenic mediators (Tacke and Weiskirchen, 2012). Several other targets were proposed that are mainly part of the oxidative or the inflammatory signaling. Molecular targets include nuclear factor-kappa B (NF-κB), tumor growth factor beta (TGF-β) and several inflammatory mediators (Popov and Schuppan, 2009). However, to protect against the development of fibrotic complications associated with the various hepatic injury, a long-term use of the medicaments is necessary. This fact limits the use of chemical products and gives a profound advantage to natural products.

In this regard, natural products have been suggested to combat human diseases including liver fibrosis. Crocin is one such nutraceutical isolated from the stigma of *Crocus sativus* commonly known as saffron which belongs to family *Iridaceae*. It is a common dietary colorant carotenoid with 14 free active functional OH groups. It has been reported to possess enormous therapeutic value. Clinically, saffron has shown antidepressant (Moshiri et al., 2006) and anti-Alzheimer activities (Akhondzadeh et al., 2010) as well as improvement of retinal function (Piccardi et al., 2012). Experimentally, crocin has shown anxiolytic (Pitsikas et al., 2008), antioxidant (El-Beshbishy et al., 2012), neuroprotective (Tamaddonfard et al., 2013), anti-inflammatory (Nam et al., 2010), hematoprotective and genoprotective (Hariri et al., 2011) and gastroprotective (El-Maraghy et al., 2015) activities. Further, crocin has been reported to ameliorate carbon tetrachloride-induced liver toxicity (Bahashwan et al., 2015) and inhibit stress-induced rise in serum transaminases in rats (Bandegi Ahmad et al., 2011). It protects against chemical insults as patulin and morphine (Boussabbeh et al., 2016; Salahshoor et al., 2016). At pharmacological and high doses, crocin did not exhibit marked damages to major organs of body and no mortality was seen by crocin in mice (Hosseinzadeh et al., 2010).

In a rat model of arthritis, crocin could inhibit the augmented serum levels of matrix metalloproteinases as well as several inflammatory mediators as TNF-α, NF-κB, and interleukins (IL) 1β and IL-6 (Hemshekhar et al., 2012). Crocin's cytoprotective and anti-apoptotic effects in PC-12 cells have been reported to involve antagonism of TNF-α-induced expression of latent interleukin-1-converting enzyme (LICE), Bcl-XS, and Bcl-XL mRNAs. In addition, crocin suppressed the TNF-α-induced activation of caspase-3 (Soeda et al., 2001). Mechanistically, cytoprotective and anti-apoptotic activities of crocin in mammalian cells have been shown to involve reactive oxygen species (ROS)-mediated endoplasmic reticulum pathway (Boussabbeh et al., 2015). Therefore, the aim of current work was to investigate the potential antifibrotic effect of crocin against thioaetamide-induced liver fibrosis in mice and elucidate the possible underlying mechanisms.

2. Materials and methods

2.1. Chemicals

Crocin and thioacetamide were purchased from Sigma–Aldrich, St. Louis, MO, USA. Other chemicals were of the highest grade commercially available.

2.2. Animals

Experiments were performed on 25–35 g male SWR mice, supplied by the Animal Breeding Laboratory, King Fahd Medical Research Center. Animals were acclimatized for 7 days before the start of experiment in our animal facility. They were maintained on a 12-h light–dark cycle. Room temperature was kept at 22 ± 2 °C. Animal handling and experimental protocol were approved by the Unit of Biomedical Ethics, Faculty of Medicine, King Abdulaziz University (Reference # 157-14).

2.3. Experimental protocol

Mice were randomly divided into five groups (8 animals per group) and treated for six consecutive weeks. Group # 1 was considered as control group and given saline i.p. twice weekly. Group # 2 was given thioacetamide (100 mg/kg, i.p.), twice weekly to provoke liver fibrosis. Group # 3 was given both of thioacetamide (100 mh/kg, i.p.) and crocin (25 mg/kg). Group # 4 was given thioacetamide with crocin (100 mg/kg). Group # 5 was given thioacetamide with the positive standard "silymarin" (100 mg/kg). Crocin and silymarin treatments were given by oral gavage every day till day 42. Blood was withdrawn from the retro-orbital plexus. Then, mice were sacrificed. Liver from each animal was rapidly dissected out, washed and homogenized using phosphate-buffered saline (PBS; 50 mM potassium phosphate, pH 7.5) at 4 °C; producing a 20% homogenate. In addition to homogenates, representative liver specimens were kept in RNAlater storage solution (Sigma–Aldrich, St. Louis, MO, USA) for RNA extraction. Liver homogenates and liver tissues for RNA extraction were kept at −80 °C till time of analyses. Representative liver tissues were kept in 10% formalin-saline for histopathological and immunohistochemical examination.

2.4. Assessment of liver functions

Activities of serum ALT and AST, serum levels of TB, TC and TG were determined colorimetrically using commercially available kits (Biodiagnostic, Cairo, Egypt).

2.5. Histopathological examination

Liver tissues were fixed in 10% neutral buffered formalin for 24 h. This was followed by immersion in tap water and serial dilutions of alcohol. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thickness by slide microtome. Tissue sections were then processed and stained with hematoxylin and eosin for histopathological examinations as previously described (Bancroft and Layton, 2013).

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