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Crocin potentiates antioxidant defense system and improves oxidative damage in liver tissue in diabetic rats

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ABSTRACT

Background: Diabetes-induced oxidative stress has an essential role in hepatic dysfunction. The current study was aimed to potentiate the impact of crocin treatment on the anti-oxidant defenses system of hepatic tissue following un-controlled hyperglycemia.

Methods: Male Wistar rats were randomly divided into four experimental groups as normal, normal treated, diabetic and diabetic treated (n = 6). Diabetes was induced by a single intravenous dose of streptozotocin into tail vein (40 mg/kg). Treated animals received crocin daily for 8 weeks intraperitoneally (40 mg/kg). At the end of the 8th week, animals were sacrificed and liver tissues were collected. After tissue preparation, malondialdehyde (MDA), nitrate and glutathione (GLT) contents and also catalase (CAT) and superoxide dismutase (SOD) enzymes activities were evaluated in all experimental animals.

Results: Un-controlled diabetes weakened anti-oxidant system by decreasing SOD and CAT enzymes activities and increasing MDA production. Crocin potentiated anti-oxidant defense system by increasing SOD and CAT enzymes activities and improved oxidative damage by lessening nitrate content and MDA production in hepatic tissues of diabetic animals.

Conclusion: Crocin maybe a potential therapeutic candidate against diabetes-induced hepatic dysfunction by attenuating oxidative damage in the hepatic tissue.

1. Introduction

Oxidative stress is involved in the development of many pathophysiological states [1]. This pathological condition occurs when free radicals are overproduced and overcome intrinsic antioxidant defense system (ADS) [2]. In physiological conditions, oxygen metabolites are mainly produced through oxidative phosphorylation reactions either enzymatically (by NADP(H) oxidase or xanthine oxidase enzymes) or non-enzymatically (by redox active compounds) [3]. Mitochondrial respiratory chain electrons contribute to reactive oxygen species (ROS) generation [3]. These free radicals have different physiological roles in gene expression, signaling processes and cellular defense against extrinsic invader pathogens.

In pathological conditions, e.g. during un-controlled hyperglycemia, free radicals are overproduced due to the activation of several pathways like glyceraldehyde autoxidation, methylglyoxal glycation, protein kinase C (PKC) activation, hexosamine pathways and oxidative phosphorylation process [4]. Furthermore, ADS attenuation in tissues during diabetes is a significant contributor to free radical overload and development of oxidative stress [5,6].

There is a large body of evidence demonstrating that free radicals exert undesirable effects in different tissues [[10],7]. Oxidative stress is associated with cardiovascular, renal, neurodegenerative and inflammatory diseases [5]. One of the susceptible tissues to oxidative stress is liver [3]. Studies have shown that the main sources of free radicals in hepatic tissue are mitochondria and cytochrome P450 enzymes [3]. It has been reported that oxidative stress is related to many forms of hepatic complications [3] such as alcoholic and non-alcoholic fatty liver diseases [8,9], hepatic encephalopathy [10], fibroproliferative diseases [11,12], and various forms of hepatitis

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[13,14].

Free radicals exert their undesirable effects in affected cells either directly via impairing biological molecules or indirectly via producing toxic substances like malondialdehyde (MDA) [5]. Different elements of ADS in hepatic tissue include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GLT) that contribute to the modulation of oxidative balance and prevention of oxidative damages [7]. However, diabetes is known to dramatically weaken ADS leading to oxidative stress [6]. Therefore, readjusting the redox state in hepatic tissue in diabetic milieu is a promising preventive and therapeutic strategy.

Crocin is a bioactive constituent of both Saffron (*Crocus Sativus* L.) and *Gardenia* plants which has beneficial effects in various tissues [15]. This water-soluble beta-carotene has free radical scavenging activity and is recognized as a potent herbal antioxidative agent [15,16]. Recent evidence suggests that crocin has desirable effects in hepatic tissue. El-Beshbishy et al. in 2012 reported that crocin protects hepatic tissue against beryllium chloride-induced oxidative damage [17]. Further recent studies emphasized on the antioxidative properties of crocin against oxidative stress induced by chronic stress [18], cisplatin [19] and carbon tetrachloride [20]. Beneficial effects of crocin in other tissues during diabetes have also been confirmed [21,22]. However, there has been little evidence about the possible effect of crocin on hepatic ADS potency and hyperglycemia-induced oxidative stress. Therefore, in the current study we investigated the possible antioxidative effects of crocin during uncontrolled hyperglycemia.

2. Methods

2.1. Animals

Male Wistar rats (200–220 g) were purchased from the Pasteur institute (Iran). The animals then were kept in standard polyester cages (2 rats per each cage) at standard temperature (22 ± 2 °C) and humidity (%55 ± 5) with 12 h light/dark cycle and free access to water and standard rodents food. Animals were randomly divided into four groups (n = 6) of control, normal treated, diabetic and diabetic treated.

2.2. Ethical considerations

All protocols of animal study were approved by the institutional animal ethics committee of the Baqiyatallah University of Medical Sciences, which follows the NIH Guidelines for the care and use of experimental animals.

2.3. Diabetes induction

Streptozotocin (STZ) was purchased from Sigma Aldrich and dissolved in cold saline. Diabetes was induced by an intravenous tail vein injection of STZ (40 mg/kg). 72 h later, blood samples were obtained from rat's tail to measure blood glucose by glucometer (Bionime, Swiss). Animals with blood glucose above 400 mg/dL were randomly divided into two diabetic groups (treated and non-treated).

2.4. Treatments

Crocin was purchased from Sigma Aldrich (USA), and then dissolved daily in distilled water. Two treatment groups (control and diabetic) were treated by crocin (40 mg/kg/day; intraperitoneally) for 8 weeks.

2.5. Sampling

At the 56th day, animals were sacrificed to remove liver tissues for assaying malondialdehyde (MDA), nitrate (Nitrate), catalase (CAT), superoxide dismutase (SOD), and glutathione (GLT) levels. Blood samples were directly collected from heart and serum was immediately separated by centrifugation (3000 rpm for 15 min)

2.6. Blood glucose assay

Serum glucose was determined using available commercial kits (Pars-Azmoon, Iran).

2.7. Tissue preparation

Liver tissue samples (500 mg) were weighed and then homogenization medium (phosphate buffer (0.1 mol, pH = 7.4) was added. After tissue homogenization on ice by electric homogenizer, samples were centrifuged (20 min at 4 °C and 4000 rpm) and supernatants were removed as the cytosolic extract of liver. Finally, supernatants were stored at −80 °C for biochemical evaluations.

2.8. Nitrate content assay

The nitrate in the 'liver cytosolic extract' as a major index of nitrous free radicals was assayed by the colorimetric reaction according to the Griess method [23]. 0.1 ml of cytosolic extract was deproteinized by adding 0.2 ml of zinc sulfate solution and centrifuged for 20 min at 4000 rpm and 4 °C to separate supernatant. 0.05 ml sulfanilamide (0.01%) and 0.05 ml *N*-[1-naphthyl] ethylenediamin di-hydrochloride (NED, 0.01%) were incubated at 37 °C for 30 min in a dark place. Then, the absorbance of solution was determined at the wavelength of 540 nm. Nitrite concentration was determined using a standard curve generated from the absorbance of each sodium nitrate solution. Finally, the nitrate content was expressed as μg/mg protein.

2.9. SOD activity

The SOD enzyme activity, as a main component of ADS in hepatic tissue, was determined using the Winterbourn method [24] which is developed based on SOD enzyme ability to inhibit the reduction of nitro-blue tetrazolium by superoxide. Potassium phosphate buffer (pH 7.8, 0.067 M) was added to 0.1 M EDTA containing 0.3 mM sodium cyanide, 1.5 mM nitro-blue tetrazolium and 0.1 ml of sample. Then, 0.12 mM riboflavin was added to each sample to trigger the reaction followed by incubation for 10 min. The sample optical absorbance was recorded at 560 nm in 5 min on a spectrophotometer. The amount of enzyme required to produce 50% inhibition was taken as 1 U and results were expressed as U/mL.

2.10. CAT activity

CAT enzyme activity as another main antioxidative element in the hepatic tissue was evaluated according to the Aebi method [25]. Reaction mixture containing 0.85 ml potassium phosphate buffer (50 mM, pH 7.0) and 0.1 ml homogenate, was incubated for 10 min at room temperature. Reaction was activated by addition of 0.05 ml H_2O_2 (30 mM prepared in potassium phosphate buffer 50 mM, pH 7.0), then decline of optical absorbance was recorded for 3 min at 240 nm. Enzyme activity was expressed as U/mL.

2.11. Assessment of GLT content

GLT content was calculated using the Tietz method [26]. Cellular protein content was precipitated by addition of 5% sulfosalicylic acid, then removed by centrifugation (2500*g* in 10 min). GLT content in supernatants was assayed as follows: 100 μl of protein-free supernatant, 800 μl of 0.3 mM Na2HPO4 and 100 μl of 0.04% 5-5'-dithiobis [2- nitrobenzoic acid] in 0.1% sodium citrate were mixed. The 5-5'-dithiobis [2-nitrobenzoic acid] absorbance was recorded at 412 nm for 5 min. A standard curve for GLT was0020performed and sensitivity of measurement was determined to be between 1 and 100 μM. The level of