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Modulatory effects of curcumin, silybin-phytosome and alpha-R-lipoic acid against thioacetamide-induced liver cirrhosis in rats

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ABSTRACT

Liver cirrhosis is the final consequence of a progressive fibrotic process characterized by excessive collagen deposition and destruction of the normal liver architecture. This study aimed to investigate the protective effects of curcumin, silybin-phytosome and alpha-R-lipoic acid against thioacetamide-induced cirrhosis. Male rats were allocated into five groups of which one group received saline and served as normal control. Animals from groups 2–5 were treated with thioacetamide administered intraperitoneally at a dose of 200 mg/kg 3 times per week for 7 weeks. Group 2 was left untreated while groups from 3 to 5 were given a daily oral dose of curcumin, silybin-phytosome or alpha-R-lipoic acid simultaneously with thioacetamide. Increases in hepatic levels of malondialdehyde (MDA) and protein carbonyls (Pr Co) associated with thioacetamide administration were partially blocked in those groups receiving supplements. Glutathione (GSH) depletion, collagen deposition, matrix metalloproteinase-2 (MMP-2) activity, transforming growth factor-β1 (TGF-β1) level as well as α-smooth muscle actin (α-SMA) and heat shock protein-47 (HSP-47) gene expressions were also decreased in response to supplements administration. Serological analysis of liver function and histopathological examination reinforced the results. In conclusion, the present study highlights the antioxidant and the antifibrotic potentials of these supplements against chronic liver diseases caused by ongoing hepatic damage.

1. Introduction

Cirrhosis is a complex phenomenon characterized by progressive fibrosis and disruption of the entire liver architecture [1]. Typical mechanisms of liver fibrosis can include those involving continuous insult to hepatocytes and Kupffer cells followed by transformation of hepatic stellate cells (HSCs) into myofibroblast like cells [2]. These cells are characterized by increased cellular proliferation, excessive deposition of extracellular matrix (ECM), mainly collagen of types I and III [1] and *denovo* expression of α-smooth muscle actin (α-SMA) [3]. Additionally, transforming growth factor-β1 (TGF-β1), heat shock protein-47 (HSP-47) and matrix metalloproteinases (MMPs) are involved in the processing, secretion and the accumulation of procollagen leading to cirrhosis as well as failure of liver function [4].

Thioacetamide (TAA) is widely used to induce liver cirrhosis in rats and the resulting disease is similar to human cirrhosis [5]. TAA is metabolically activated to thioacetamide sulfoxide and further to thioacetamide-S,S-dioxide, an unstable reactive metabolite that initiates necrosis by covalently binding to liver macromolecules [6]. Accumulating evidence has indicated that oxidative stress plays a critical role in the activation of HSCs as well as in the progression of fibrosis and cirrhosis [7].

Curcumin (CUR) is the most active constituent extracted from Turmeric rhizome (*Curcuma longa*) [8]. It possesses powerful antioxidant activity [9] allowing it to modulate cellular redox signaling

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; ANOVA, analysis of variance; ARE, antioxidant response element; AST, aspartate aminotransferase; CYP2E1, cytochrome P450 2E1; DHLA, dihydrolipoic acid; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; GGT, γ-glutamyl transferase; GSH, glutathione; H&E, hematoxylin and eosin; HMG-COA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; HRP, horseradish peroxidase; HSCs, hepatic stellate cells; HSE, heat shock promoter element; HSF1, heat shock factor 1; HSP-47, heat shock protein 47; Keap1, Kelch-like ECH-associated protein 1; LDH, lactate dehydrogenase; MDA, malondialdehyde; MMP-2, matrix metalloproteinase-2; MMPs, matrix metalloproteinases; M-MuLV, moloney murine leukemia virus; Nrf2, nuclear factor-erythroid-2-related factor 2; PAI-1, plasminogen activator inhibitor; PC, phosphatidylcholine; PPAR-γ, peroxisome proliferator-activated receptor-γ; Pr Co, protein carbonyls; RT-PCR, real time polymerase chain reaction; TAA, thioacetamide; TGF-β1, transforming growth factor-β1; TG, triglycerides; α-SMA, α-smooth muscle actin.

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pathways. Curcumin has been also reported to exhibit beneficial therapeutic effects in chronic liver disease owing to its hepatoprotective activities [10]. It could suppress the gene expression of TGF-β receptors and alleviate hepatic fibrosis by inhibiting HSCs activation as well as collagen α1(I) gene expression [11].

Silybin is the predominant and most active component of silymarin, which is a mixture of flavanolignans extracted mainly from dried milk thistle seeds (*Silybum marianum*) [12]. Silybin is known as an anti-hepatotoxic agent [13] due to its putative antioxidant, anti-inflammatory and anti-proliferative properties based on the modulation of specific signalling pathways, transcription factors and gene expressions of many proinflammatory and profibrogenic mediators [14,15].

Interestingly, water-soluble phytoconstituents can be converted into a lipid-compatible complex known as phytosome. A phytosome increases the bioavailability of active constituents due to its enhanced capacity to cross the lipid-rich biomembranes and reach circulation [16]. In silybin-phytosome (Ultrathistle, ULT), phosphatidylcholine (PC) is the lipid-phase substance employed to make silybin lipid-compatible. PC is not merely a passive "carrier", but is itself a bioactive nutrient with documented clinical efficacy against multiple liver diseases [17].

Alpha-lipoic acid (α-LA) is a naturally occurring dithiol compound synthesized from octanoic acid in the mitochondrion. It functions as a cofactor for mitochondrial α-ketoacid dehydrogenases. There is growing evidence that dietary supplied LA exerts meritorious physiological effects, not only in experimental animals but also in humans [18]. Having one chiral center, LA can therefore exist in both R- and S-enantiomeric forms; however some studies have indicated that the R-enantiomer is more effective than its S-counterpart in inducing various biological activities [19]. The oxidized (LA) and reduced dihydrolipoic acid (DHLA) create a potent redox couple called "universal antioxidant" capable to regenerate several antioxidants [20]. LA is also described as a potent modulator of various inflammatory signaling pathways and as a detoxification agent [21].

In view of that, this study is attempted to provide an update on the hepatoprotective effects and the undisclosed antifibrotic mechanisms of curcumin, ultrathistle and α-R-lipoic acid against TAA-induced cirrhosis in rat model.

2. Materials and methods

2.1. Chemicals

Thioacetamide (TAA) and curcumin were purchased from Sigma–Aldrich chemicals co. (St. Louis, MO, USA). Ultrathistle® (silybin-phytosome) and α-R-lipoic acid® were obtained from Natural Wellness Co., USA. TAA, as a chemical inducer, and the three supplements were either dissolved or suspended in sterile normal saline before use.

2.2. Animals

Male Wistar albino rats, weighing 140–180 g, were obtained from the National Institute for vaccination's farm, Egypt. They were housed in standard plastic cages in an environmentally controlled condition, and allowed to receive pelleted standard rat chow diet and *ad libitum* water throughout the experimental period. The study was carried out according to the European Communities Council directive of 2010 (2010/63/EU) and was approved by the Ethical Committee for Animal Experimentation at Faculty of Pharmacy, Cairo university.

2.3. Study design

One week after acclimatization, rats were randomly divided into:

Group 1 (control group): this group served as saline-treated control.
Group 2 (TAA group): animals were treated with TAA administered i.p. on alternative days (200 mg/kg 3 times per week) for 7 weeks [22].
Group 3 (TAA + CUR group): animals were treated with TAA, as previously mentioned, and an oral daily dose of curcumin (400 mg/kg) [23].
Group 4 (TAA + ULT group): animals were treated with TAA and an oral daily dose of ultrathistle (400 mg/kg) [24].
Group 5 (TAA + α-R-LA group): animals received TAA and 200 mg/kg/day of α-R-lipoic acid, given orally [25].

After 7 weeks, animals were fasted over night. They were then sacrificed; blood and tissue samples were harvested for subsequent analyses.

2.4. Blood and tissue collection

Serum was separated and used for the quantification of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), albumin, total cholesterol, triglycerides (TG), total and direct bilirubin as well as TGF-β1 levels. The liver was isolated and then divided into three portions. One portion was homogenized in 10 volumes of ice-cold double distilled water using a Teflon homogenizer (Glas-Col homogenizer, Terre Haute, USA). An aliquot of the 10% homogenate was mixed with an equal volume of ice-cold 100 mM phosphate buffer pH 7.4 , centrifuged at 11,000*g* at 4 °C for 20 min using a Sorvall Combiplus ultracentrifuge (Du Pont Company, USA) and the resultant supernatant was taken for the determination of protein carbonyls (Pr Co) levels. Other aliquots were mixed with 7.5% sulfosalicylic acid and 2.3% KCl solutions, centrifuged at 600*g* at 4 °C for 15 min, and the resulting supernatants were used for the determination of glutathione (GSH) and malondialdehyde (MDA) contents, respectively. The second portion of the liver was used for the determination of α-SMA and HSP-47 gene expressions using real time polymerase chain reaction (RT-PCR). The remaining portion was kept in 10% formaldehyde, and then embedded in paraffin for subsequent histopathological and immnunohistochemical examinations.

2.5. Biochemical estimation

2.5.1. Determination of liver function

Spectrophotometric diagnostic kits provided from Biodiagnostic (Egypt), Quimica Clinica Aplicada S.A. (Spain) and Stanbio (USA) were used.

2.5.2. Determination of Pr Co level

Protein carbonyls were measured using the spectrophotometric method for the detection of protein hydrazones following reaction with dinitrophenylhydrazine. Results were expressed as nanomoles of carbonyls per milligram of protein. Because about 10–15% of proteins are lost in the reaction procedure, the protein levels were quantified in the final pellets by reading the absorption at 280 nm. The amount of proteins was calculated from a bovine serum albumin standard treated in the same way [26].