The Hepatoprotective Effects of Chrysin in Animal Model of Non-Alcoholic Fatty Liver Disease. The Impact on Angiotensin Converting Enzyme 2/Angiotensin 1-7/ Mas Axis

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the build-up of extra fat in liver due to insulin resistance. Oxidative stress, inflammation and the activation of classical arm of renin angiotensin system (RAS) contributes to NAFLD. However, the alternative arm of RAS named angiotensin (Ang) converting enzyme 2 (ACE2)/Ang 1-7/Mas receptor counteracts the classical axis and improves hepatic lipid metabolism This study aimed to investigate the impact of chrysin, a potent antioxidant flavonoid, on this protective axis in NAFLD. **Methods:** Rats were treated daily as follows: normal controls, NAFLD model (20% fructose in drinking water), treated groups (25 and 50 mg/kg chrysin concomitantly with fructose). After eight weeks, rats were reweighed, serum levels of liver enzymes, glucose and triglycerides (TG) as well as hepatic TG, oxidative stress, inflammatory markers, were determined using colorimetric and ELISA kits. Protein levels of ACE2, Ang 1-7 and Mas were determined using western blotting. Structural changes were detected by H&E staining. **Results:** High fructose resulted in significant weight gain, hepatocyte degeneration, inflammatory infiltration and accumulation of lipid droplets. Serum TG & glucose and hepatic levels of TG, lipid peroxides, and inflammatory markers were markedly elevated, while levels of ACE2, Ang 1-7 and Mas were significantly reduced. Chrysin (25 and 50 mg/kg) significantly attenuated these abnormalities with a prominent effect of 50 mg/kg on improving the levels of glucose, TG, ACE2, Ang 1-7 and Mas. **Conclusions:** Chrysin could be used for efficient protection from NAFLD via enhancing ACE2/Ang 1-7/Mas axis.

Background

NAFLD is the hepatic manifestation of metabolic syndrome, with an increasing prevalence in people with obesity up to 60–80% [1]. Multiple etiologies of NAFLD have been proposed, among them RAS plays an essential role. The classical arm of RAS; ACE/Ang II/AT1 receptor is implicated in the development and progression of NAFLD via the stimulation of insulin resistance, de novo lipogenesis, mitochondrial dysfunction, oxidative stress, and pro-inflammatory cytokine production as well as the activation of hepatic stellate cells to trigger fibrogenesis [2]. However the alternative axis, composed of ACE2/Ang-7/Ras receptor has been appears to exert anti-proliferative, anti-inflammatory, anti-fibrotic and anti-thrombotic actions [3]. It is has been also reported that activation of the ACE2/Ang-(1–7)/Mas axis led to improved hepatic insulin resistance, and that loss of ACE2 activity worsens liver fibrosis in chronic liver injury models, and administration of recombinant ACE2 shows therapeutic potential [4]. Cao et al [5] reported that, Ang-(1–7)/ACE2 ameliorated hepatic steatosis, and that deletion of ACE2 aggravates the development of these conditions in mice. Furthermore, they demonstrated that overexpression of ACE2 improved fatty liver in db/db mice. These results indicated that this axis could be a promising target for the protection from NAFLD. Therefore, study aims to to confirm the role of ACE2/ Ang-(1–7)/Mas axis in the protection against NAFLD. This goal was achieved by using dimethazine aceturate (DIZE) which is a commonly used as ACE2 activator in many animal models [6]. It is now thought that high intake of fructose is one of major causes of chronic metabolic diseases, including obesity, diabetes and NAFLD [7].

Objectives

Our aim is extended to investigate the role of chrysin, an anti-oxidant flavonoid in ameliorating high fructose - induced NAFLD via activation of ACE2/Ang 1-7/Mas axis.

METHODOLOGY

Animals and Study Design

Wistar rats (150-200 g) were divided randomly into 6 groups (8 rats/each) and treated daily for 8 weeks as follows: Group 1: normal control received carboxy methyl cellulose (CMC, 0.5% in normal saline), Group 2: drug control received 50 mg/kg chrysin (dissolved in 0.5% CMC solution) by oral gavage. Groups 3 was the model group given 20% fructose in drinking water + equivalent volume of 0.5% CMC (the vehicle of chrysin), Groups 4, 5 were treated respectively with 25 and 50 mg/kg chrysin dissolved in 5% CMC, concomitant with 20% fructose. Group 6: rats received DIZE (15 mg/kg, s.c., dissolved in normal saline) + equivalent volume of 0.5% CMC concomitant with 20% fructose. DIZE was used as a reference ACE2 activator.

Sample preparation and histological studies: At the end of experiment and after overnight fast, rats were weighed, sacrificed by decapitation and serum was separated for the assay of liver function test including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and for the assay of serum glucose and triglycerides (TG). Liver was separated and divided into parts as following: One part is kept in -80 °C to prepare tissue homogenate and used to for the assay of hepatic TG, oxidative stress and inflammatory markers. Second part was kept in liquid nitrogen for western blot analysis of Ang II, ACE2, Ang-1-7 and Ras. Third part was fixed in 10% formalin for histological study using hematoxylin and eosin (H&E) stain.

Assay of oxidative stress and inflammatory markers: Hepatic levels of malondialdehyde (MDA) and reduced glutathione (GSH) were assayed using colorimetric kits according to manufacturers' instructions. The inflammatory markers including tumor necrosis- α (TNF-α), interleukin-6 (IL-6) and nuclear factor kappa B (NF-κB), were estimated by ELISA assay.

Assay of hepatic TG:

The hepatic TG content was determined using a triglyceride colorimetric kit (Wako Pure Chemical) after extraction of the lipid fraction from frozen liver specimens by the method of Bligh and Dyer [8].

Molecular study: The protein levels of AngII, ACE2, Ang-1-7 and Ras were determined using western blot analysis

Statistical Analysis: The data were analyzed by GraphPad Prism 4.0 using one-way ANOVA followed by Tukey-Kramer test. All data were expressed as mean ± SEM. P<0.05 was considered statistically significant.

RESULTS

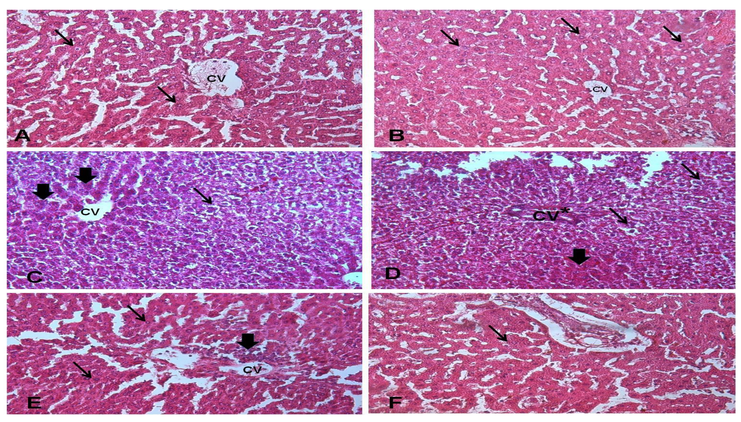

Figure 1: Light micrograph of liver sections stained with H&E. (A) & (B) Liver sections from normal control and chrysin control, respectively, showing normal liver lobular architecture, normal hepatocytes (thin arrow) and vasculature (CV). (C) and (D) Liver section from rat received 20% fructose showing hepatocyte degeneration (thin arrow), vascular congestion (CV*) ballooning and fatty degeneration of the heaptocytes, and aggregations of inflammatory cells (thick arrow). (D) & (E) Liver sections from rat received 20% fructose and concomitantly treated with chrysin at 25 and 50 mg/kg, respectively, showing marked decrease of fat deposition and inflammatory cells (Thick arrow) with improvement of liver architecture and normalization of heptocytes (thin arrow).

RESULTS

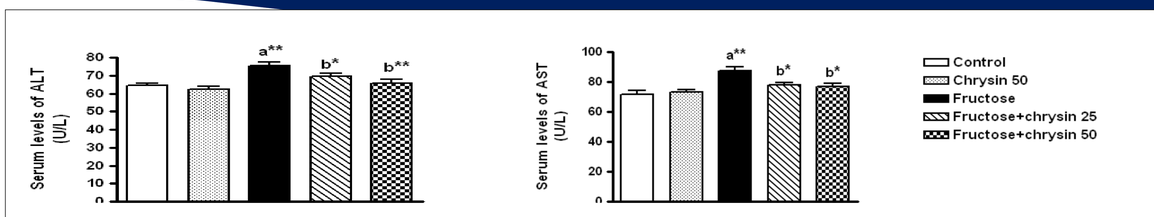

Figure 2: The effect of 25 and 50 mg/kg chrysin on liver enzymes in high fructose-induced NAFLD. Values are expressed as Mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; *** P < 0.001, ** P < 0.01, * P < 0.05

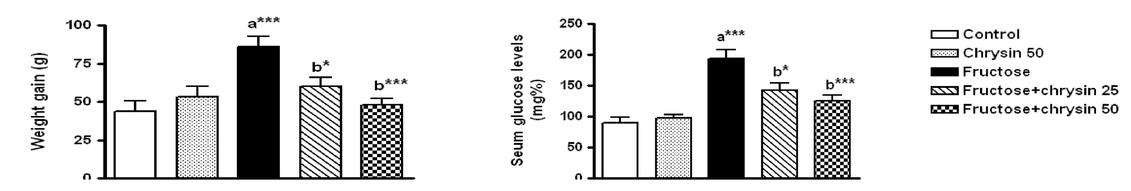

Figure 3: The effect of 25 and 50 mg/kg chrysin on body weight gain an d fasting glucose levels in high fructose-induced NAFLD. Values are expressed as Mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; *** P < 0.001, * P < 0.05

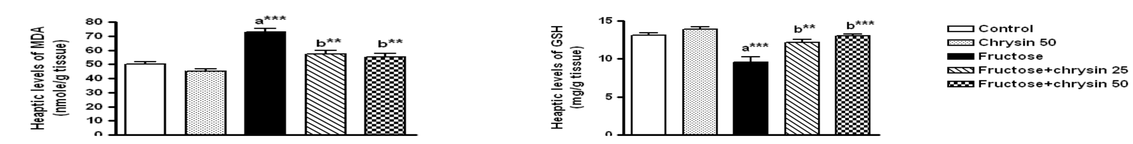

Figure 4: The effect of 25 and 50 mg/kg chrysin on heaptic levels of malondialdehyde (MDA) and reduced glutathione (GSH) in high fructose-induced NAFLD . Values are expressed as mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; *** P < 0.001, ** P < 0.01

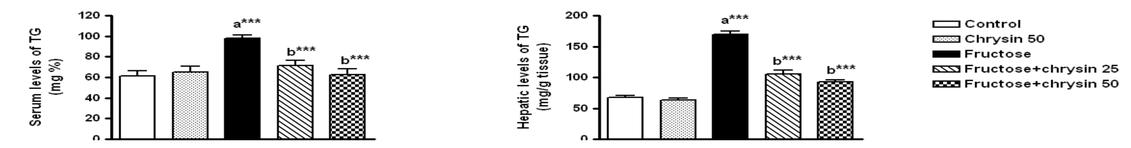

Figure 5: The effect of 25 and 50 mg/kg chrysin on serum and heaptic levels of triglycerides (TG) in high fructose-induced NAFLD . Values are expressed as mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; *** P < 0.001

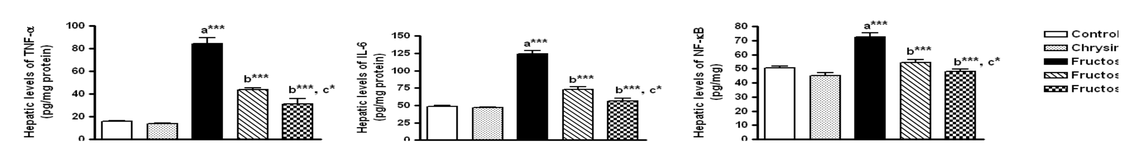

Figure 6: The effect of 25 and 50 mg/kg chrysin on hepataic levels of tinflammatory markers includingg tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and nuclear factor kappa B (NF-kB) in high fructose-induced NAFLD. Values are expressed as mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; c: significantly different from Fructose + chrysin 25;

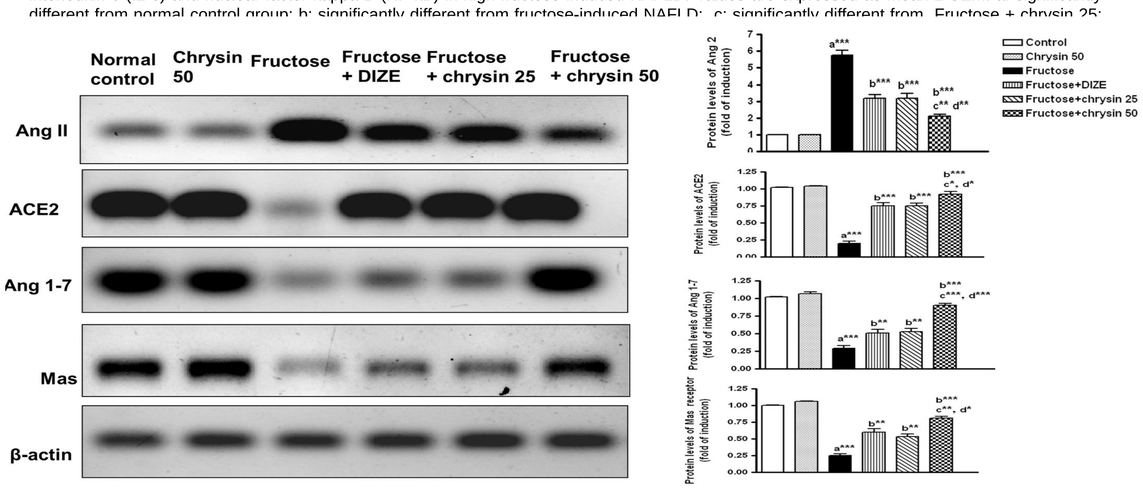

Figure 7: Representative immunoblots and quantitative analysis of the protein levels of Ang II, ACE2, Ang-1-7 and Ras in liver tissues of controls, NAFLD, DIZE and chrysin- treated groups. Values are expressed as mean ± SEM. a: significantly different from normal control group; b: significantly different from fructose-induced NAFLD; c: significantly different from Fructose + chrysin 25, d: significantly different from Fructose + DIZE; *** P < 0.001, ** P < 0.01, * P < 0.05.

CONCLUSION

Our demonstrated that daily supplementation with chrysin at either dose 25 or 50 mg/kg body weight could efficiently protect from NAFLD. Depletion of the augmented levels of Ang II as well as up-regulating the components of the protective axis of RAS including ACE2, Ang 1-7 and Mas could be a potential mechanism, particularly with the dose 50 mg/kg.

Discussion

- The current research provides an evidence of the protective role of Chrysin in NAFLD. Further research to fully elucidate different mechanisms of Chrysin, such as its effect on Sphingosine Pathway and insulin resistance is strongly recommended
- Also the possible implication of ACE2/Ang 1-7/Mas axis is documented in this research, further study of other ACE2 activators and their potential activity against NAFLD is a valuable step toward better understanding the role of this axis and its possible clinical applications.

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