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The Efficacy of Licorice Root Extract in Decreasing Transaminase Activities in Non-alcoholic Fatty Liver Disease: A Randomized Controlled Clinical Trial

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This study was performed to investigate the effects of licorice on non-alcoholic fatty liver disease (NAFLD). In this double blind randomized clinical trial, 66 patients were divided into case and control groups. All patients had elevated liver enzymes and had increased liver echogenicity (lipid accumulation) on sonography. The case group was treated with one capsule containing 2 g aqueous licorice root extract per day for 2 months while the control group was treated in the same manner with a placebo. Weight, body mass index (BMI) and liver transaminase levels were measured for each patient before and after the study. In the case group, the mean alanine aminotransferase (ALT) level decreased from 64.09 to 51.27 IU/mL and the aspartate aminotransferase (AST) level decreased from 58.18 to 49.45 IU/mL, which were statistically significant ($p < 0.001$ and $p < 0.001$). But in the control group, a drop in the ALT and AST levels was not statistically significant. The BMI difference before and after the study was not statistically significant in both groups. Despite the significant drop in liver enzymes following administration of licorice root extract, it is recommended that further studies that include histological examination are necessary.

Keywords: non-alcoholic fatty liver disease; NAFLD; NASH; licorice root; glycyrrhizin.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common condition with an increasing incidence. Progression to cirrhosis and potential for mortality or requirement of liver transplantation is observed in some cases (Chande *et al.*, 2006). Non-alcoholic fatty liver disease is the most common disorder related to the liver in developed countries. It is estimated that 20–40% of the Western population (Chitturi *et al.*, 2007) and 5–35% of the population of Pacific and Asian countries (Shen *et al.*, 2003; Amarapurkar *et al.*, 2007) are afflicted with NAFLD. The highest incidence of this disease is reported between 40 and 49 years of age (Ludwig *et al.*, 1980; Ikai *et al.*, 1995; Noguchi *et al.*, 1995).

One type of progressive NAFLD is known as non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis, hepatocellular carcinoma (HCC), liver dysfunction and ultimately to death (Day and James, 1998; Chande *et al.*, 2006; Hajaghamohammadi *et al.*, 2008). The major risk factors for NAFLD – central obesity, type 2 diabetes mellitus, dyslipidemia and metabolic syndrome – are common in western societies (Ikai *et al.*, 1995; Sonsuz *et al.*, 2000; Wanless and Lentz, 1990). The prevalence is increased in men, older individuals and those with hypertension, obesity or diabetes. Non-alcoholic steatohepatiti was confirmed by biopsy in 30% of ultrasound-positive patients (Williams *et al.*, 2011).

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In a study of 4009 administrative officers in Shanghai, Shen *et al.* (2003) found the overall prevalence of fatty liver was 12.9% and the prevalence of fatty liver was positively correlated to several risk factors, including male, ageing (> 50 yr), hyperlipidemia, impaired glucose tolerance/diabetes mellitus, hypertension and overweight/obesity. Ultrasound is still the first option for diagnosis, but its accuracy depends on the operator and the patient's features. Computed tomography can detect hepatic fat content, but only at a threshold of 30%, and it involves ionizing radiation. Magnetic resonance (MR) spectroscopy is probably the fastest and the most accurate method of detecting fat, but it is expensive and the necessary software is still not easily available in most magnetic resonance imaging (MRI) units (Roldan *et al.*, 2008). A combination of serum biomarkers and radiological modalities may one day provide the best diagnostic approach for patients with NAFLD, and potentially replace the necessity for liver biopsy in most patients (Mishra and Younossi, 2007). Despite increasing prevalence of NAFLD, its exact molecular and cellular mechanisms remain obscure and effective therapeutic strategies are still limited. Inhibition of free fatty acid (FFA)-associated hepatic toxicity represents a potential therapeutic strategy (Wu *et al.*, 2008).

There is no proven effective therapy for NASH, although modification of risk factors such as obesity, hyperlipidemia and poor diabetic control is generally recommended. Weight loss is the only therapy with reasonable evidence supporting benefit. Weight loss and increased physical activity can lead to sustained improvement in liver enzymes, histology, serum insulin

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levels and quality of life in patients with NASH (Hickman *et al*., 2004; Promrat *et al*, 2010). The observation that vitamin E decreases oxidative stress provided a rationale for its evaluation in patients with NASH. Initial observational data suggested a benefit on aminotransferase levels in patients with NASH, leading to a number of controlled trials (Lavine, 2000; Sanyal *et al*., 2010, Lavine *et al*., 2011). Several hypoglycaemic agents continue to be evaluated for the treatment of NASH. Although metformin has no effect on histology, it could still be beneficial in reduction in serum levels of lipids and glucose (Hauteland *et al*., 2009). Rosiglitazone has a considerable antisteatogenic effect in the first year of treatment but no additional benefit with longer treatment despite a sustained effect on insulin sensitivity and transaminase levels. This shows that improving insulin sensitivity is insufficient in NASH, and that other targets of therapy for NASH should be detected (Ratziu *et al*., 2010).

Orlistat is a gastrointestinal lipase inhibitor used in the treatment of obesity and type 2 diabetes mellitus. A pilot randomized controlled trial in patients with NASH found a significant reduction in fatty liver as assessed by ultrasound (Zelber-Sagi *et al*., 2006). Socha *et al*. (2009) in a systematic review on different drugs such as vitamin E, metformin, pioglitazone, ursodeoxycholic acid, probucol and carnitine, could not indicate a drug of choice for NAFLD.

Licorice root has been used as a dietary supplement for stomach ulcers, bronchitis and sore throat, as well as infections caused by viruses, such as hepatitis (Angelico *et al*., 2007). Licorice grows in various countries, including Iran, China, Turkey, Russia and Italy. Glycyrrhizin (GL), the major bioactive component of licorice root extract, has a variety of pharmacological properties, including antiinflammatory, antioxidant and immune-modulating activities. Glycyrrhizin has been used to treat hepatitis to reduce liver inflammation and hepatic injury; however, the mechanism underlying its antihepatic injury property is still poorly understood (Aoki *et al*., 2007; Wu *et al*., 2008). Hence, this study was designed to investigate the effects of aqueous licorice root extract on liver enzymes in NAFLD.

MATERIALS AND METHODS

This double blind study was conducted in the Gastrointestinal and Liver Clinic of Qazvin (central IRAN). The inclusion criteria were presence of NAFLD confirmed by sonography and presence of elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). All tests and ultrasound processes were performed at the same institution in order to avoid personal errors.

A complete history of drug and alcohol consumption was taken and autoimmune hepatitis and viral markers were checked for all patients. Patients with a history of diabetes, hypertension, ischaemic heart diseases or alcohol abuse, or those positive for autoimmune hepatitis and viral markers, were excluded from the study.

To evaluate the effects of licorice root extract on NAFLD, patients were divided into two groups randomly (by using a random number table). The case group was treated with one capsule containing 2 g aqueous licorice root extract alone (20% glycyrrhizin) per day for 2 months, while the control group was treated in the same manner with a placebo (2 g starch). Both drugs were in the form of a capsule. The placebo was completely similar to the active drug in respect of the shape, colour and package, and all its ingredients were identical to the main drug except for the licorice active extract, which was absent in the placebo.

Blood samples were sent to an external laboratory and the testers were not informed about the groups of patients. Weight, body mass index (BMI) and liver transaminase levels were measured for each patient before and after the study. Data were analysed by SPSS® (v. 11.5). Comparisons of BMI, liver enzymes and other numerical variables were assessed by independent samples *t*-test and between-group comparisons were done using paired *t*-test.

RESULTS

After approval by the ethics committee and informing the subjects regarding the project and obtaining informed consent, 66 patients including 38 men (57.6%) and 28 women (42.4%) were enrolled in the study and all completed it. No patients in the two groups dropped out or were excluded from the study during the research period. This may be due to excluding patients with a higher risk of licorice side effects such as patients with hypertension and ischaemic heart diseases. Eighteen men (54.50%) and 15 women (45.50%) in the case group and 20 men (60.60%) and 13 women (39.40%) in the control group were studied. Mean age of case and control groups was 39.9 and 40.5 yr, respectively. Age ranged from 28 to 52 yr in the case group, and from 21 to 56 yr in the control group. Mean weight was 81.25 and 80.50 kg in the case and control groups respectively ($p > 0.05$). Mean BMI of the case group was 30 kg/m^2 before administration of the drug, and decreased to 29.20 kg after the treatment ($p > 0.05$). Mean BMI in the control group before drug administration was 29.10 kg/m^2, which increased to 29.22 kg after the treatment ($p > 0.05$). Mean serum ALT level in the case group was 64.09 and 51.27 IU/mL before and after treatment with licorice ($p < 0.001$; Fig. 1), whereas it was 66.90 and 62.77 IU/mL in the control group before and after administration of the placebo ($p > 0.05$; Fig. 1). The mean serum AST level in the case group was 58.18 and 49.45 IU/mL before and after treatment with licorice ($p < 0.001$; Fig. 2), while it was 57.86 and 54.81 IU/mL in the control group before and after treatment with the placebo ($p > 0.05$; Fig. 2).

DISCUSSION

Central to the pathogenesis of NAFLD is insulin resistance. Chitturi and colleagues noted that 98% of patients with NASH in their study had insulin resistance (Chitturi *et al*., 2002). Insulin resistance increases lipolysis and delivery of free fatty acids to the liver (Bernard *et al*., 2000) or decreases synthesis of apolipoproteins and potentially leads to decreased export of triglycerides out of the liver (Chalton *et al*., 2002). In one study, patients with NASH were more likely than patients with

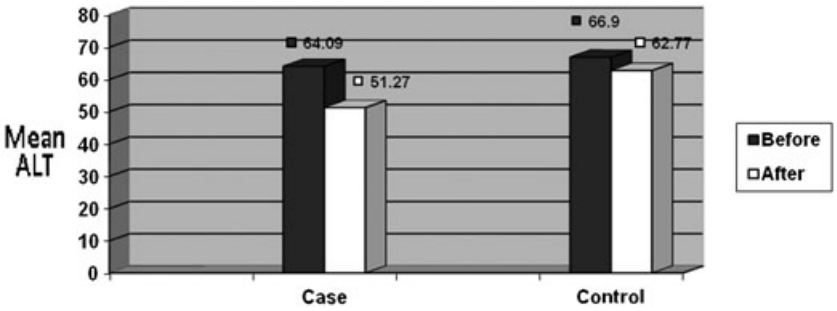

Figure 1. Alanine aminotransferase (ALT) levels before and after treatment with licorice root extract in the case and control groups.

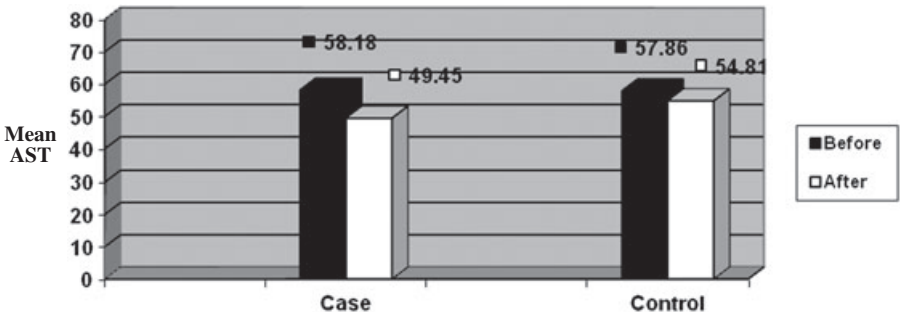

Figure 2. Aspartate aminotransferase (AST) levels before and after treatment with licorice root extract in the case and control groups.

simple steatosis to fulfill criteria for the metabolic syndrome (88% versus 53%; Choudhury and Sanyal, 2004). There are several factors or second hits such as oxidative stress and other cytokines that promote inflammation and fibrosis (George *et al*., 1998).

To our knowledge, no data regarding NAFLD treatment with licorice root extract have been published so far, but licorice root extract is one of the drugs used in the treatment of chronic liver diseases in Iranian Traditional Medicine, with no serious side effects. Some studies have shown that glycyrrhizin could improve lipoprotein lipase expression, insulin sensitivity and serum lipid in rats (Lim *et al*., 2009; Eu *et al*., 2010). Considering our findings and taking into account the importance of liver disease and availability of herbal medicines in Iran, usage of this drug in patients with NAFLD might be feasible.

The duration and the recommended dosage should be studied in the future research. In our study, the effect of licorice on liver histology (liver biopsy) and patient's prognosis was not studied because it is quite invasive. Other limitations that should be considered in this study are that it is not clear whether the level of reduction in transaminases has clinical significance (i.e. does it means that the NAFLD is improving), and it is not clear if transaminases are the best markers of NAFLD as the epidemiological study by Shen *et al*. (2003) did not find that ALT was a good predictor of NAFLD. Therefore, future studies could include more detailed tests of liver function and liver histology in addition to measures of liver enzymes. Based on the results of this study, licorice root extract appears safe and may be considered in the treatment of elevated liver enzymes in NAFLD.

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Conflict of Interest

The authors declare no conflict of interest.

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