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Short Communication

A STUDY ON THE PROTECTIVE EFFECT OF *SILYBUM MARIANUM* EXTRACT ON HEPATIC ISCHEMIA-REPERFUSION INJURY

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Abstract

The objective of the study was to study the protective effect of *Silybum marianum* extract on hepatic ischemia-reperfusion injury. Rats were randomly divided into five groups; namely *Silybum marianum* extract high-, medium-, and low-dose protection groups, model group and control group. Hepatic ischemia-reperfusion injury model was prepared. Serum or plasma AST, ALT, MDA, TNF-α, IL-1β, IL-6 levels were measured. The results revealed that after liver injury, AST, ALT, MDA, TNF-α, IL-1β, and IL-6 levels significantly increased in succession, showing significant differences. We concluded that inflammatory cytokines participate in liver injury and that *Silybum marianum* extract can reduce the production of inflammatory cytokines, and thus can have a protective effect on hepatic ischemia and reperfusion.

Keywords: *Silybum marianum*, hepatic ischemia-reperfusion injury, protective effect

Introduction

Silybum marianum is the dried ripe fruit of Compositae plant *Silybum marianum (L.)* Gaertn. It is bitter in taste, cool in nature, and enters the liver and gallbladder meridians. It can clear away heat and toxic substances, and soothe liver and gallbladder. It is used in the treatment of liver and gallbladder damp-heat, hypochondriac pain, and jaundice. It has extensive pharmacological effects such as hepatoprotective, antitumour, hypolipidemic and antiatherogenic, myocardial protective, cerebral ischemia protective, aldose reductase inhibitory, antituberculotic, and antioxidant effects (Ma & Hu., 1998; Jong et al., 2004; Ji & Zhao., 2012; Biswajit et al., 2012). Silymarin is the total extract of medicinal active ingredients from *Silybum marianum* fruit, in which the main ingredients are silybin, isosilybin, silydianin, and silychristin (Liu, 2009). As a conventional hepatoprotective drug, silymarin has been widely used in the field of treatment of liver diseases for its significant efficacy and low toxicity (Wang et al., 2005; Nirav et al., 2010). This experiment was carried out to determine whether *Silybum marianum* extract has a protective effect on hepatic ischemia-reperfusion, providing a reference basis for clinical medication.

Materials and Methods

Animals

50 SD rats, 8 weeks old, half male and half female, weighing 180±20 g were provided by the Central South University (Animal certificate is zxp-2008), and used for the experiment.

Reagents and main instruments

Silybum Marianum was identified by Mr Xing Li of Central South University. The specimen was placed at the lab centre of university. ALT, MDA, TNF-α, IL-1β, and IL-6 kits are all from Nanjing Jiancheng Bioengineering Institute. Glacial acetic acid, anhydrous ethanol, rotary evaporator (Shanghai Yarong Biochemical Instrument Factory), and centrifuge, 37 ℃ constant temperature water bath were also used.

Preparation of *Silybum marianum* extract

500 g of powder was weighed and placed in a 5000 ml round-bottomed flask. 2500 ml of 80% ethanol solution was added to it before it was soaked for 30 min. It was then extracted through heat reflux for 3 times using electric heating mantle. Then the 3 filtrates were combined, and ethanol was recovered in a rotary evaporator. The obtained paste was dissolved in distilled water to prepare a solution with a concentration of 1 g/ml, which was placed in a 4 ℃ refrigerator for later use.

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Animal grouping, pharmacological preconditioning, and HIRI model preparation

50 SD rats were randomly divided into five groups; namely the blank group (open exposure of liver), model group, and treatment groups (high-, medium-, and low-dose groups). All rats were adaptively fed for one week. Starting from the second week, preconditioning was performed every day in accordance with the following methods: blank group and model group were intragastrically administrated 1 ml of normal saline per day, and treatment groups were intragastrically administrated 1 ml of *Silybum marianum* extract (high-, medium-, and low-doses) per day. All experimental procedures were approved by the Animal Research Ethics Committee of Xinxiang Medical College University

Hepatic ischemia-reperfusion injury (HIRI) model establishment

Healthy SD rats, after being fasted for 12 h, were intraperitoneally injected with 10% chloral hydrate (40 mg/kg) to induce anaesthesia, fixed in the supine position. Then their abdomens were approached via the ventral midline, and ligaments around the liver were separated to expose hepatic portal. Abdominal cavities in sham injury group were closed. In treatment group and model group, hepatic arteries, portal veins and common bile ducts were occluded with non-invasive vascular clamp to cause the liver to enter into the ischemic state. Abdominal cavities were closed. 1 h after ischemia, abdomens were cut open, and vascular clamps were loosened to restore blood perfusion. Liver colour changed from dark red to bright red; that is, the animal HIRI model was established, followed by the abdominal cavity closure. 1 h after perfusion, abdomens were cut open. 4 ml of blood was sampled from the inferior vena cava of the rat in each group, and serums were centrifugally separated for later use.

ALT, AST, TNF-α, IL-1β, IL-6 measurement

TNF-α, IL-1β, and IL-6 were measured by enzyme-linked immunosorbent assay method (ELISA). MDA was measured by thiobarbituric acid method and all measurements were performed in strict accordance with the instructions. ALT was measured by conventional biochemical method; that is, by automatic biochemical analyser.

Statistical methods

Statistical analysis was performed using SPSS 15.0 software. Dose data were expressed as mean ± standard deviation, and significant test for comparison among control group and other groups was done by Dunnett test.

Results

Compared with the sham injury group, ALT, AST, TNF-α, IL-1β, and IL-6 values were all significantly increased in model group, and the differences were statistically significant ($p<0.01$). ALT, AST, TNF-α, IL-1β, and IL-6 values were all significantly reduced in treatment groups when compared with the model group, and the differences were statistically significant ($p<0.01$) (Tables 1 and 2). The reduction effect was more evident with the increase of drug concentration, indicating that the *Silybum marianum* extract can reduce the production of inflammatory cytokines, inhibit AST, ALT, etc., and thus has a certain protective effect on hepatic ischemia-reperfusion.

Table 1: Comparison of serum AST, ALT, and MDA among rats in each group

Group	AST (U/L)	ALT (U/L)	MDA (μmol/L)
Sham injury group	94.55±16.93	104.49±16.73	3.36±0.48
Model group	749.82±63.85$^{\#\#}$	783.92±54.62$^{\#\#}$	12.95±0.62$^{\#\#}$
Treatment group (high-dose)	395.16±45.93**	412.84±32.97**	6.07±0.56**
Treatment group (medium-dose)	521.93±34.26**	503.54±29.07**	8.94±0.34**
Treatment group (low-dose)	602.73±51.64**	623.53±31.87**	10.05±0.47**

$^{\#\#}$ P<0.01 Comparison with the sham injury group; ** P<0.01 Comparison with the model group

Table 2: Comparison of serum TNF-α, IL-1β, IL-6, and IL-8 levels among rats in each group

Group	TNF-α (ng/L)	IL-1β (ng/L)	IL-6 (ng/L)
Sham injury group	22.78±0.24	140.87±54.62	170.57±20.93
Model group	95.09±2.41$^{\#\#}$	239.64±61.93$^{\#\#}$	265.19±32.15$^{\#\#}$
Treatment group (high-dose)	38.03±3.51**	160.23±37.22**	190.17±34.63**
Treatment group (medium-dose)	52.10±2.81**	187.44±42.98**	223.28±27.92**
Treatment group (low-dose)	78.44±3.46**	204.58±28.91**	240.16±31.37**

$^{\#\#}$ P<0.01 Comparison with the sham injury group; ** P<0.01 Comparison with the model group

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Discussion

Hepatic ischemia-reperfusion injury is a kind of severe clinical pathophysiological change, which is usually divided into the ischemia period and reperfusion period. The major pathophysiological manifestations in the ischemia period only include the structural changes in membrane potential and metabolic acidosis caused by changes in intracellular calcium ion concentration. But changes in liver function are not significant, and the damage is less severe. While in the reperfusion period, injury is significantly exacerbated. Liver function indices change significantly, even giving rise to related complications. A large number of studies have found that the early-stage reperfusion injury after hepatic ischemia is divided into two phases. The first phase, which spans the first 6 hours from the start of reperfusion, is known as the acute phase. The major pathophysiological changes are activation of kuPffer cells, release of a large number of inflammatory mediators, as well as the generation of reactive oxygen species. From the 6th hour, the injury enters the second phase, which is referred to as the sub-acute phase. Main manifestations are suppressed kupffer cell function, consumption of inflammatory mediators, neutrophil aggregation and other manifestations of exacerbated liver cell damage. Therefore, inflammatory mediators play a key role in hepatic ischemia-reperfusion injury, of which TNF-α level increases. Moreover, it may be an important initiation factor for many injury factors. TNF-α has important regulatory and mediation effect on the immune response and inflammatory response, and can stimulate monocyte macrophages and other cells to secrete IL-1, IL-6 and other inflammatory factors. The liver is an important place for synthesis and removal of IL-6. Therefore, the level of IL-6 can directly reflect the strength of liver function (Wu et al., 2006).

Currently, it is believed that the serum aminotransferase levels are the best indicator for determining the degree of acute liver injury, because it is expressed in a high level in the serum only after the damage of liver cells, and is less interfered by other factors. ALT exists in the cytoplasm, and AST exists in the cytoplasm and mitochondria. When mitochondria and cell membrane are damaged, these two enzymes are released into the blood, thus making their serum concentrations increase rapidly. A research suggested that the serum aminotransferase levels of liver function can directly reflect the HIRI degree, the severe the injury, the higher the aminotransferase levels (Yuan et al., 2008). In this experiment, rat serum ALT and AST levels are determined via the rat liver ischemia-reperfusion model. The experimental results show that the ALT, AST levels in ischemia-reperfusion group are significantly higher than the normal group, indicating that the ischemia-reperfusion can cause acute liver injury, while the *Silybum marianum* extract high-, medium, and low-dose groups can all significantly reduce the serum ALT, AST levels ($p<0.01$) after the ischemia reperfusion, indicating that the *Silybum marianum* extract can significantly reduce the ischemia reperfusion-induced liver injury.

MDA is a metabolite of lipid peroxidation, as it can form conjugate crosslinking and polymeric structure with the membrane phospholipids, protein molecules, etc. It can change the basic characteristics of membranes, make the protein lose activity, and thus cause cell death. Therefore, determination of MDA level can reflect the degree of lipid peroxidation, and indirectly reflect the tissue content of oxygen free radicals as well as the degree of cell damage caused by the attack of oxygen free radicals (Tan & Pu., 2008). In this experiment, the level of MDA in serum and liver tissue of rats are determined via the rat hepatic ischemia-reperfusion model. The results reveal large amounts of oxygen free radical generation in the hepatic ischemia reperfusion group. But MDA contents in *Silybum marianum* extract high-, medium-, and low-dose groups are all significantly lower than that of the hepatic ischemia reperfusion group ($P<0.01$), indicating that the *Silybum marianum* extract has a significant anti-lipid peroxidation effect. Hence, it can alleviate liver damage caused by ischemia and reperfusion through the anti-free radical actions.

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