**Name of journal: *World Journal of Gastroenterology***

**ESPS Manuscript NO: 27643**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

**Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat**

Jiang W *et al.* Hepatoprotective effects of lycopene

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**Author contributions:** Hai X guaranteed the entire study; Jiang W carried out the whole experiment; Guo Mh participated in the design of the study,performed the statistical analysis, and drafted the manuscript; Guo Mhand Hai X edited and reviewed the manuscript; all authors read and approved the final manuscript.

**Institutional review board statement:** The study was reviewed and approved by Harbin Medical University Institutional Review Board, Harbin, China.

**Institutional animal care and use committee statement:** All procedures involving rat in this manuscript were reviewed and approved by the Institutional Animal Care and Use Committee on the Ethics of Animal Experiments of Harbin Medical University (HMU, Protocol Number: 20150301).

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

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**Manuscript source:** Unsolicited manuscript

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**Received:** June 8, 2016

**Peer-review started:** June 13, 2016

**First decision:** July 29, 2016

**Revised:** August 15, 2016

**Accepted:** October 10, 2016

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To evaluate the hepatoprotective effect of lycopene (Ly) on non-alcoholic fatty liver disease (NAFLD) in rat.

***METHODS***

A rat model of NAFLD were first established using a high-fat diet for 14 weeks. Sixty-five rats were randomly divided into normal group, model group and Ly treatment groups. Alanine transferase (ALT), aspartate transaminase (AST), triglycerides (TG), total cholesterol (TC) in serum and low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), free fatty acid (FFA), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) in liver tissue were evaluated, respectively. While the hepatoprotective effect were also confirmed by histopathological analysis, and the expression of TNF-α and cytochrome P450 (CYP) 2E1 in rats liver were determined by immunohistochemistry (IHC) analysis.

***RESULTS***

A significantly decrease was observed in the levels of serum AST (2.07-fold), ALT (2.95-fold), and the blood lipid TG (2.34-fold), TC (1.66-fold) in the dose of 20 mg/kg Ly-treated rats (*p* < 0.01), compared to the model group. Pretreatment with 5, 10 and 20 mg/kg of Ly significantly raised the levels of antioxidant enzyme SOD with a dose-dependent manner to 90.95 ± 9.56, 109.52 ± 11.34 and 121.25 ± 10.68 (*p* < 0.05, *p* < 0.01) as compared with the model group. Similarly, the levels of GSH were significantly increased (*p* < 0.05, *p* < 0.01) in after the Ly-treated. While, pretreatment with 5, 10 and 20 mg/kg of Ly significantly reduced MDA amount by 30.87, 45.51 and 54.49% in the liver homogenate, respectively (*p* < 0.01). Ly-treatment group was signiﬁcantly decreased the levels of lipid products LDL-C (*p* < 0.05, *p* < 0.01), improved HDL-C level and significantly decreased the content of FFA, compared to the model group (*p* < 0.05, *p* < 0.01). Furthermore, Ly-treated group also exhibited a down-regulated TNF-α and CYP2E1 expression, decreased the infiltration of liver fats and reversed the histopathological changes in a dose-dependent manner (*p* < 0.05, *p* < 0.01).

***CONCLUSION***

This study suggests that Ly has a protective effect on NAFLD, down-regulated expression of TNF-α and CYP2E1 may be one of the action mechanism for Ly.

**Key words**:Lycopene; Non-alcoholic fatty liver; Hepatoprotective; Antioxidant; Cytochrome P450 2E1

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**Core tip:** Lycopene (Ly), a phytochemical belonging to carotenoid family, is a red colored pigment, apolar and acyclic carotenoid. The present study was designed to evaluate the possible hepatoprotective effect of Ly on non-alcoholic fatty liver disease (NAFLD) in rat. It is the first time to examine the effects of Ly on the therapy of NAFLD, and the down-regulated expression of TNF-α and CYP2E1 may be one of the action mechanism for Ly.

Jiang W, Hai X, Guo Mh. Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. *World J Gastroenterol* 2016; In press

**INTRODUCTION**

Non-alcoholic fatty liver disease (NAFLD) is one of the causes of [fatty liver](https://en.wikipedia.org/wiki/Fatty_liver), occurring when fat is deposited ([steatosis](https://en.wikipedia.org/wiki/Steatosis)) in the [liver](https://en.wikipedia.org/wiki/Liver) due to causes other than [excessive alcohol use](https://en.wikipedia.org/wiki/Alcoholism). NAFLD is considered to covers a spectrum of liver diseases including simple steatosis, non-alcoholic steatohepatitis (NASH), liver ﬁbrosis, liver cirrhosis and hepatocellular carcinoma (HCC)[1,2]. Ninety percent of patients with NAFLD have closely related with one or more of the following risk factors: hypertension, dyslipidemia, elevated triglyceride levels, obesity, insulin resistance, metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease[3]. Currently, the percentage of people with NAFLD is approximately 20% worldwide and 25% in Western countries, making it one of the most dominant causes of chronic liver disease affecting both adults and children[4]. NAFLD is more common in patients with severe diabetes and obesity, mortality and disease evolution to liver ﬁbrosis or liver cirrhosis is increased in old people with NAFLD[5]. Recently, the “two-hit” theory has been accepted as a popular mechanism, although the cause of NAFLD was not clearly elucidated[6]. Furthermore, there is no any speciﬁc drug can be available, and no drug has currently been tested in clinical phase III trials. Therefore, there is no speciﬁc therapy can be ﬁrmly recommended to the patients with NAFLD[7].

Lycopene (Ly), a phytochemical belonging to carotenoid family, is a red colored pigment, acyclic and apolar carotenoid[8]. It is abundantly found in red colored vegetables and fruits such as tomatoes, papaya, gac fruit, pink grape-fruit, pink guava, carrots and watermelon, with the concentrations ranging from 9 to 42 mg/kg depending on the variety[9]. Ly displays a range of unique and distinct biological properties owing to its acyclic structure, hydrophobicity and large array of conjugated double bonds. Recently, diverse studies have been reported that lycopene has a powerful antioxidant both in vitro and in vivo against the oxidation of proteins, lipids, and DNA, and also has the potential of quenching singlet oxygen 100 times more efﬁciently than vitamin E and 125 times more than glutathione[10]. Furthermore, even at low oxygen tension, it can also scavenge peroxyl radicals, inhibiting the process of lipid peroxidation[11]. It is the most efﬁcient quencher of singlet oxygen among all naturally occurring carotenoids [11], and recently it has been in great demand as a food additive and a natural antioxidant. Additionally, lycopene also exhibited a potent neuroprotective, anti-inﬂammatory, anti-proliferative, maintaining normal cell metabolism, cognition enhancing properties, regulating blood lipid metabolism and so on[12-16]. Therefore, with this background, we would like to investigate the possible beneﬁcial effects and the possible action mechanism of Ly on NAFLD in rats.

**MATERIALS AND METHODS**

***Materials and reagents***

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), free fatty acid (FFA), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) kits were obtained from Nanjing Jiancheng Bioengineering Institute (China). Protein assay kit was from Zhongshan Institute of Biotechnology (Beijing, China).

Mouse anti-TNF-α, rabbit anti-cytochrome P4502E1 (CYP2E1), horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, HRP-conjugated goat anti-rabbit IgG antibodies were provided by Proteintech Group, Inc. (Chicago, USA).

Lycopene (> 95%) was purchased from North China Pharmaceutical Co., Ltd., China.

The high fat diet (HFD: 88% basic feed + 10% lard + 2% cholesterol) was prepared in our lab.

***Animals***

Male Wistar rats body weight 150 ± 10 g were obtained from Experimental Animal Center of Harbin Medical University, China. Six rats were kept in one polyacrylic cages on a 12 h day/night cycle and quarantined for one week before the experiments. All animals were housed under standard controlled conditions (temperature: 24 ± 1℃, humidity: 50% ± 5% and 12 h light/dark cycle), free access to food and water, and received human care according to National Institutes of Health Guidelines of United States (National Research Council of United States, 1996) and related ethical regulations of Harbin Medical University. Animals were fasted for 12 h before sampling of material.

***Experimental design***

After acclimatization for one week, 65 Wistar male rats were randomized into 2 groups. Group 1 (normal group) were raised with normal feed (*n* = 12), Group 2 (model group) were raised on high fat feed (HFD: 88% basic feed + 10% lard + 2% cholesterol) (*n* = 53) for consecutive 8 weeks. From the 9th week, all the survival rats in the model group were further randomly divided into a model group and three doses of Ly treatment groups, which were given Ly with a dose of 5, 10 and 20 mg/kg/d (*n* = 12), respectively. Model group was continued to be fed with the HFD for 6 weeks as before, and Ly groups were administered orally and continued to be fed with the HFD for 6 weeks as before.

Rats were sacrificed by cervical dislocation at the end of the experiment, blood samples of all rats were harvested for serum biochemical markers assay. The fresh liver obtained was weighed to calculate liver coefficient (Liver coefficient % = liver weight/body weight × 100). The right liver lobe was fixed in 10% formalin to prepare paraffin sections and the rest was stored at -80℃ for the other assays.

***Serum biochemical markers assay***

Serum was collected from blood after the centrifugation at 3000 rpm for 10 min, 4°C. Serum ALT, AST, TG and TC were detected using commercial kits according to the manufacturer’s instructions by a multifunctional biochemistry analyzer Olympus AU600 (Olympus, Tokyo, Japan). The observation absorbance of ALT and AST were read at 505 nm and the enzyme activity was calculated as U/L. The observation absorbance of TG and TC was read at 510 nm and the content was calculated as mmol/L.

***Measurement of MDA formation in lipid peroxidation***

Liver homogenate (10%, w/v) was prepared by homogenizing the liver tissue in 150 mmol/L Tris-HCl buffered saline (pH 7.2) with a polytron homogenizer. The level of MDA in liver tissues was measured at 532 nm with a spectrophotometer (Hitachi U-2001) following the kit protocol from Jiancheng Biological Engineering Institute (Nanjing, China). The data are expressed as nmol/mg protein of liver tissue.

***Measurement of antioxidant and antioxidant enzyme activity***

SOD and GSH activity were determined by commercial kit from Jiancheng Biological Engineering Institute (Nanjing, China) following the protocol provided by manufacture. The observation absorbance of SOD reaction was read at 550 nm and the data are expressed as U/mg protein, while GSH reaction was read at 420 nm and the enzyme activity was calculated as mg/g protein.

***Measurement of liver LDL-C, HDL-C and FFA activity***

LDL-C, HDL-C and FFA in liver tissue were measured by commercial kit from Jiancheng Biological Engineering Institute (Nanjing, China) following the protocol provided by manufacture. The observation absorbance of LDL-C, HDL-C and FFA reaction was read at 546 nm and the data are expressed as mmol/L.

***Histopathological observation***

Liver specimens were fixed overnight in 10% formaldehyde buffer, then embedded in paraffin and cut into 5 µm thickness according to the routine procedure. The sections stained with hematoxylin and eosin (H&E) for routine histopathological examination, and examined under a light microscope (Olympus BX-50 Microscope, Leica Microsystems, Germany) at 200 × magnification for the degree of hepatic steatosis and photographed.

***Immunohistochemistry analysis of hepatic TNF-α and CYP2E1***

Paraffin-embedded sections (5 μm) were mounted on glass slides, then deparaffinized, incubated in 3% H2O2, 10 min to quench endogenous peroxidase activity. The sections were stained with mouse anti-TNF-α antibody and rabbit anti-CYP2E1 antibody at 4°C overnight respectively, after blocking with normal goat serum for 20 min. Then incubation with HRP-conjugated goat anti-mouse and HRP-conjugated goat anti-rabbit antibody at 37 °C, 30 min, respectively. The biding sites of the antibody were visualized by incubation with DAB-H2O2 at room temperature, 10 min. Images were taken at original magniﬁcation of 200 × (Olympus BX-50 Microscope, Japan and Leica DMIL, Leica Microsystems, Germany).

***Statistical analysis***

Data were expressed as mean ± SD and all statistical comparisons were made by means of a one-way ANOVA test followed by Dunett’s *t*-test. *p* < 0.05 and < 0.01 were considered statistically significant.

**RESULTS**

***Effects of Ly on body weight and liver coefficient***

After 8 weeks HFD feeding, the body weights of rats in model group had a significant increase compared rats that of in control groups (*p* < 0.01, Figure 1A). While, after Ly-treated for 6 weeks, the gain of the rats body weight in 10 and 20 mg/kg Ly-treated groups was lower than that of model group (*p* < 0.01, Figure 1A), which indicated that Ly treatment could inhibit the occurrence of obesity in HFD administrated rats. Furthermore, consistent with these modifications, the live coefficient was also reduced markedly in Ly-treated rats (*p* < 0.05, *p* < 0.01, Figure 1B), compared to control group.

***Effect of Ly on serum ALT and AST levels***

Serum levels of AST and ALT indirectly reflects the failure of liver function. In Table 1, serum AST (2.67-fold) and ALT (3.66-fold) activities were significantly increased after the administration of HFD as compared with the normal group (*p* < 0.01). Compared with model group, the levels of AST and ALT were significantly decreased in a dose-dependent manner after Ly treatment (5, 10 and 20 mg/kg) (*p* < 0.05, *p* < 0.01, Table 1).

***Effects of Ly on blood lipid levels***

High fat and cholesterol diet induced NAFLD provoked a markedly increment of TC and TG levels compared with normal group (*p* < 0.01, Table 1), which indicates the successful establishment of the NAFLD model in rats. However, after Ly exposure, the concentrations of both TC and TG in blood were remarkably decreased in dose-dependent manners versus NAFLD model group (*p* < 0.05, *p* < 0.01, Table 1). All of these indicated that Ly had obvious lipid-lowering effects against NAFLD.

***Effects of Ly on liver tissue SOD, GSH and MDA levels***

The levels of liver antioxidant activities of SOD and GSH were measured due to the oxidative stress exhibited in the development of NAFLD[17]. SOD and GSH could scavenge the lipid hydroperoxides, lipid peroxide radicals and other products which is a toxic metabolite of NAFLD. Therefore, our study measured the contents of SOD, GSH and MDA in liver tissue of rats. From Table 2, we can clearly see the significant difference between HFD-treated model group and the normal group, the levels of SOD and GSH were largely decreased (*p* < 0.01, Table 2) in HFD-treated group compared with that of normal group. However, pretreatment with 5, 10 and 20 mg/kg of Ly significantly raised the levels of antioxidant enzyme SOD with a dose-dependent manner to 90.95 ± 9.56, 109.52 ± 11.34 and 121.25 ± 10.68 (*p* < 0.05, *p* < 0.01, Table 2) as compared with the model group. Similarly, the levels of GSH were significantly increased by treatment with 10 and 20 mg/kg of Ly (*p* < 0.05, *p* < 0.01, Table 2).

MDA, an end-product of the breakdown of polyunsaturated fatty acids and related esters, is an important index of lipid peroxidation in many organ homogenate[17]. Administration with HFD caused a significant increase in MDA concentration (2.19-fold) when compared with the normal group (*p* < 0.01, Table 2). However, pretreatment with 5, 10 and 20 mg/kg of Ly significantly reduced MDA amount by 30.87, 45.51 and 54.49% in the liver homogenate, respectively (*p* < 0.01, Table 2).

***Effects of Ly on LDL-C, HDL-C and FFA levels in liver tissue***

Levels of lipid products were significantly increased after 8 weeks HFD feeding in model group compared to control group (*p* < 0.01, Table 3). Results showed that LDL-C was signiﬁcantly increased in model group compared with normal group (*p* < 0.01, Table 3) and dramaticly decreased in Ly-treatment group compared that of model group (*p* < 0.05, *p* < 0.01, Table 3). In contrast, HDL-C level was significantly decreased at the end of experiment, and Ly-treatment could signiﬁcantly improved HDL-C level compared with that in model group (*p* < 0.05, *p* < 0.01, Table 3). Similarly, the concentration of FFA was remarkably increased after HFD administration, and while pretreatment with Ly significantly decreased the content of FFA in a dose-dependent manners (*p* < 0.05, *p* < 0.01, Table 3).

***Histopathological changes in the liver tissue***

Observed under naked eyes, livers were deep red, moist, glossy and resilient in control group (Figure 2AⅠ), while yellow necrosis foci, grey-red, lost luster and tumescent were often found on the surface of livers in model group (Figure 2A Ⅱ). However, in Ly-treated rat, liver injury was attenuated dramatically in a dose-dependent manners (Figure 2A Ⅲ-V).

H&E stained sections are shown in Figure 2B. Under the photomicroscope, liver sections showed normal lobular architecture, liver cells with a well-preserved cytoplasm and well-deﬁned nucleus in the normal control group (Figure 2BⅠ). While in the model group, full fat vacuoles in lobule cells, infiltration of inflammatory cells, cell swelling and lipid degeneration could be seen in the central region of the lobules (Figure 2B Ⅱ). Furthermore, in Ly-treated group, inflammatory response and lipid degeneration were remarkably alleviated as compared with the model group, and the liver cell volume became smaller, the fat droplet number was reduced and the hepatic lobules were clearly delineated (Figure 2B Ⅲ-V).

***Effect of Ly on immunohistochemistry analysis of hepatic TNF-α and CYP2E1***

In the immunohistochemical analysis, liver tissue shows no TNF-α expression in normal group (Figure 3AⅠ), while HFD-model group increased the expression of TNF-α in liver (Figure 3AⅡ). After pretreatment with Ly (5, 10 and 20 mg/kg), TNF-α expression shows decrease in a dose-dependent manners, but it is still higher than normal group (Figure 3A Ⅲ-V). Quantiﬁcation of the positive expression of TNF-αshowed in Figure 3A Ⅵ. Result is showed as the mean ± SD of (*n* = 12). b*p* < 0.01 signiﬁcantly different from normal group; c*p* < 0.05 and d*p* < 0.01 signiﬁcantly different from model group, respectively.

In Figure 3B, the normal liver expressed the lowest amount of CYP2E1 (Figure 3B Ⅰ). HFD-model group significantly highly expressed CYP2E1 when compared with the controls (*p* < 0.01, Figure 3B Ⅱ). While Ly-treated group (5, 10 and 20 mg/kg) shows markedly decreased of CYP2E1 expression (Figure 3B Ⅲ-V). Quantiﬁcation of the positive expression of CYP2E1 showed in Figure 3B Ⅵ. Result is showed as the mean ± SD (*n* = 12). b*p* < 0.01 signiﬁcantly different from control group; c*p* < 0.05 and d*p* < 0.01 signiﬁcantly different from model group, respectively.

**DISCUSSION**

NAFLD is defined by hepatic fat deposition in the absence of excessive alcohol intake, which associated with the insulin resistance (IR) and metabolic syndrome[18-20]. Generally, NAFLD is defined as a concentration of hepatic TG exceeding 5% liver weight, and often exhibited a histological spectrum ranging from simple steatosis to NASH. NASH is characterized by hepatocellular damage, fibrogenesis and lobular necro-inflammation[21,22], which may evolve to hepatic cirrhosis and HCC[23,24]. Although HFD induced NAFLD animal models need a lengthy feeding period, these models are more close to human NAFLD in the pathophysiology, including induced obesity, IR and hepatic steatosis in mice or rats[25]. Emotional disorders or poor diet with the key points of blood stasis and phlegm is regarded as the etiology of NAFLD, and these etiologies are related to the organs of liver, spleen and kidney, according to the traditional medicine theory[26]. Thus, promoting blood circulation to remove meridian obstruction, reducing phlegm, removing dampness and liver-kidney-tonifying are an effective way for the treatment of NAFLD. However, at present, although tremendous effort has made in prevention of NAFLD from clinicians and researchers, there are no approved treatment drugs for NAFLD. Hence, develop and explore a novel agent to delay or reverse the pathogenesis progression in NAFLD is very important.

Ly is a natural pigment, synthesized by plants and microorganisms. Red fruits and vegetables are the most common sources of Ly, which exhibits the highest antioxidant activity among all dietary carotenoids. Furthermore, the Mediterranean dietary pattern, which include proportionally high consumption of [vegetables](https://en.wikipedia.org/wiki/Vegetable) and [fruits](https://en.wikipedia.org/wiki/Fruit) with Ly, has shown notable benefits for NAFLD patients[27,28]. Therefore, nowadays, the potential role of Ly in human health is beginning to be recognized, and the most important health benefits are hypothesized to occur through their ability to protect against oxidative damage[29,30]. In vitro studies, Ly is reported as an effective antioxidant and radical scavenger[31,32]. Ly is the most potent singlet oxygen quencher among natural carotenoids, since its high number of conjugated dienes[33], and recent studies show that Ly at least two times as active as β-carotene in protecting lymphocytes for NO2· radical induced membrane damage[34,35], and which indications that Ly is the most potent scavenger of ROS among other major dietary carotenoids[36,37]. In addition, Ly protected human LDL against photosensitized oxidative damage[32]. Thus, based on the benefits of Ly, the aim of the present study was to explore the effect of Ly in prevention of HFD induced NAFLD rat model. To our best knowledge, this is the first time to explore the potent effects of Ly on HFD-induced NAFLD rats.

In the present study, compared to normal control group, it is demonstrated that the liver coefficient and the levels of serum ALT, AST, TG and TC were signiﬁcantly increased, the levels of LDL-C and FFA in liver were markedly increased, and HDL-C were markedly reduced in HFD-induced NAFLD model group. While pretreatment with Ly, we found that Ly is able to inhibition the increment of ALT and AST, to decreased TG, TC, LDL-C and FFA levels, and to increased HDL-C levels. In addition, the histopathological changes from microscopy observation correlated with the examination of liver function. The centrilobular hepatic necrosis, ballooning degeneration, fatty change and infiltrating lymphocytes were observed in NAFLD model group. Treatment with Ly prevented these histopathological changes in rat induced with HFD. Thus, these results suggested that the inhibition of the elevation of liver function markers, obvious lipid-lowering and liver damage may related in the protective effect of Ly against HFD-induced NAFLD. Moreover, Ly enhanced the activities of SOD, increased the GSH and diminished MDA against the HFD-induced NAFLD in these animals, suggesting that the activity of antioxidant may plays a role in the mechanism of its hepatoprotective effects.

TNF-α is a central proinflammatory cytokine, which associated with a variety of physiological and pathological conditions including cytotoxicity, growth stimulation, immune-modulation and pro-inﬂammatory activity. In addition, since TNF-α is produced predominantly by the monocyte macrophage lineage in liver and the mainly population of this lineage is Kupffer cells. Thus, increased TNF-α production by activated Kupffer cells may be responsible for NAFLD. Furthermore, currently studies have displayed that inhibition of TNF-α could decrease the content of hepatic fatty storage in HFD-induced NAFLD model[38]. In our study, the effects of TNF-α in damaged liver was evaluated by IHC. Compared to the normal group, treated with HFD up-regulated the expression of TNF-α, while pretreatment with Ly down-regulated the expression of TNF-*α* compared to the HFD-model group.

The isoform 2E1 of CYP is one of the most potent microsome cytochromes to generate ROS, and it is involved in the metabolism of isoniazid and the mediation of its hepatotoxicity[39], which has been exhibited to be invariably increased in the livers of NAFLD patients[40]. In this study, the expression of CYP2E1 in HFD-model group was observed increased, while Ly treatment group showed a significant down-regulate its expression, especially in the high dose of Ly-treated group.

In conclusion, oral administration of Ly improves lipid profiles and remarkably decreased the levels of serum AST, ALT, TG and TC, alleviate the levels of liver LDL-C and FFA, increased the activities of antioxidant enzymes (GSH, SOD) and reduced the lipid peroxides in liver (MDA) in NAFLD model rats. Further, Ly-treated group also down-regulated the expression of TNF-α and CYP2E1, decreased the liver fats infiltration and improved the histopathological changes in a dose-dependent manners. The increased antioxidant enzyme levels and the decreased lipid peroxides contents are suggested to be the important mechanisms of Ly in preventing the development of liver damage induced by HFD.

**COMMENTS**

***Background***

Non-alcoholic fatty liver disease (NAFLD) is one of the causes of [fatty liver](https://en.wikipedia.org/wiki/Fatty_liver), which encompasses a spectrum of liver diseases including simple steatosis, non-alcoholic steatohepatitis (NASH), liver ﬁbrosis, liver cirrhosis and hepatocellular carcinoma (HCC). Until now, there is no any speciﬁc drug can be available, and no drug has currently been tested in clinical phase III trials. Lycopene (Ly), a phytochemical belonging to carotenoid family, is a red colored pigment, apolar and acyclic carotenoid. Ly exhibits a range of distinct and unique biological properties owing to its acyclic structure, hydrophobicity and large array of conjugated double bonds. Recent report showed that the Mediterranean dietary pattern, which include proportionally high consumption of [vegetables](https://en.wikipedia.org/wiki/Vegetable) and [fruits](https://en.wikipedia.org/wiki/Fruit) with Ly, has shown notable benefits for NAFLD patients. Thus, with this background, we would like to investigate the possible beneﬁcial effects and the possible action mechanism of Ly on NAFLD in rats.

***Research frontiers***

No any speciﬁc drug has currently been tested in clinical phase III trials for NAFLD, and there is few research on the study of hepatoprotective effects of Ly.

***Innovations and breakthrough***

It is the first time to examine the effects of Ly on the therapy of NAFLD, and the down-regulated expression of TNF-α and CYP2E1 may be one of the action mechanism for Ly.

***Applications***

This study suggests that Ly has a protective effect on NAFLD, which is very important for the development of the potent NAFLD drug.

***Terminology***

NAFLD is one of the causes of [fatty liver](https://en.wikipedia.org/wiki/Fatty_liver), deﬁned as biopsy proven hepatic steatosis. It covers a spectrum of liver diseases including simple steatosis, NASH, liver ﬁbrosis, liver cirrhosis and HCC. Recently, many NAFLD drug researches are focus on the traditional Chinese medicines.

***Peer-review***

This is an meanful study in which related to effects of “lycopene” on NAFLD rat model. This results are very important and suggest that lycopene has a protective effect on NAFLD through down-regulated TNF-α and CYP2E1 expression.

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**P-Reviewer:** Abenavol L, Lee HC **S-Editor:** Gong ZM

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

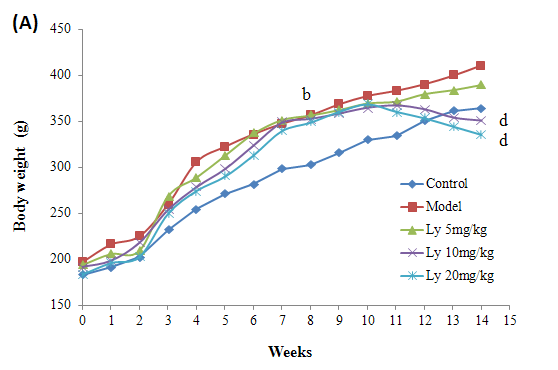
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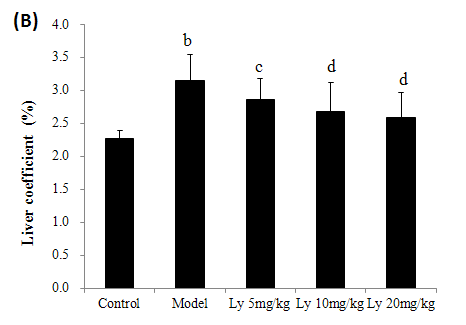
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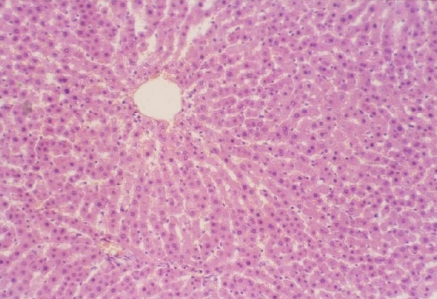
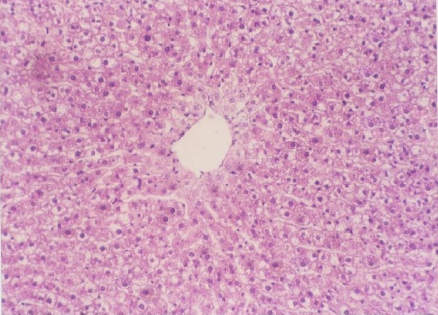
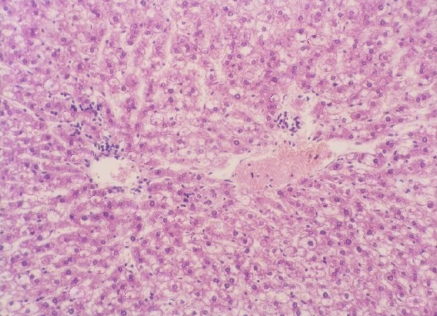
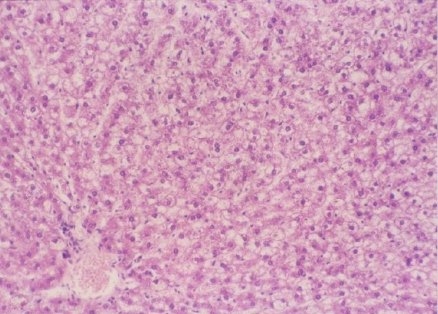
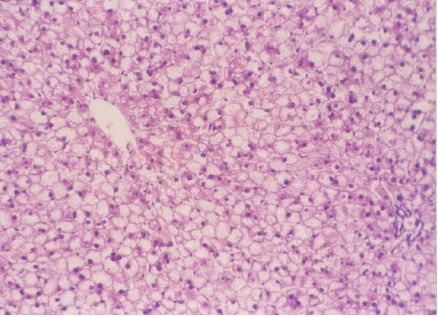
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**Figure 1** **Effects of the lycopene on body weight (A) and liver coefficient (b)**.b*p* < 0.01 *vs* control group;c*p* < 0.05, d*p* < 0.01 *vs* model group. Ly: lycopene.



**Ⅰ**

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**IV**

**V**

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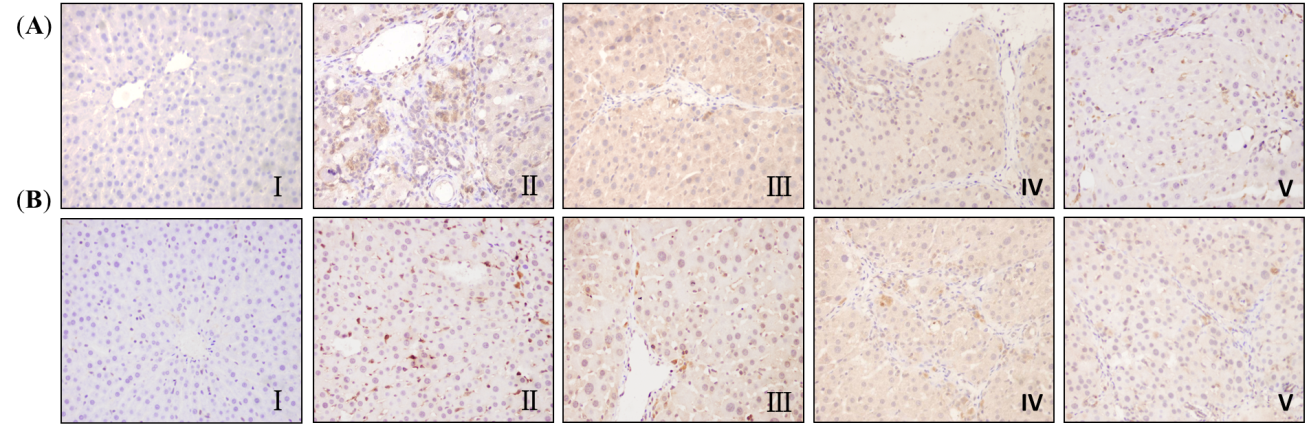
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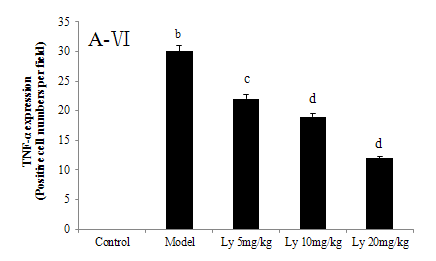
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**(A)**

**(B)**

**Figure 2** **The appearance of rat liver tissue (A) and histopathological examination by H&E (B, 200 ×).** I: Control group, II: Model group, III: Ly 5 mg/kg group, IV: Ly 10 mg/kg group, V: Ly 20 mg/kg group. Ly: lycopene.

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**Figure 3** **Representative photographs of immunological histological chemistry examination (200 ×)**. **A**: TNF-α, **B**: CYP2E1. Ⅰ: Control group, Ⅱ: Model group,Ⅲ: Ly 5 mg/kg group, IV: Ly 10 mg/kg group, V: Ly 20 mg/kg group; Ⅵ: Quantiﬁcation of TNF-α(A-VI) and CYP2E1 (B-VI) stained cells. Result is expressed as the mean ± SD of 12 rats. b*p* < 0.01 *vs* control group;c*p* < 0.05, d*p* < 0.01 *vs* model group. Ly: lycopene.

**Table 1 Effect of lycopene on serum liver function markers and blood lipid levels**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | **ALT ( IU/L)** | **AST ( IU/L)** | **TG ( mmol/L)** | **TC (mmol/L)** |
| Control | 16.72 ± 2.62 | 60.65 ± 6.28 | 0.52 ± 0.04 | 0.81 ± 0.06 |
| Model | 61.25 ± 13.55b | 162.17 ± 35.53b | 1.38 ± 0.21b | 3.04 ± 0.72b |
| Ly 5mg/kg | 30.90 ± 3.84c | 95.91 ± 13.65c | 1.02 ± 0.10 | 2.31 ± 0.24 |
| Ly 10mg/kg | 26.33 ± 2.06d | 88.53 ± 9.18d | 0.75 ± 0.06c | 2.00 ± 0.12c |
| Ly 20mg/kg | 20.77 ± 3.52d | 78.44 ± 9.79d | 0.59 ± 0.03d | 1.83 ± 0.15d |

Data are expressed as the mean ± SD (*n* = 12) in each group. b*p* < 0.01 *vs* control group;c*p* < 0.05, d*p* < 0.01 *vs* model group. Ly: Lycopene; ALT: Alanine transferase; AST: aspartate transaminase; TG: triglycerides; TC: total cholesterol.

**Table 2** **Effect of lycopene on liver antioxidant enzymes-specific activities, antioxidant and lipid peroxidation levels**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **SOD (U/mgprot)** | **GSH (mg/g prot)** | **MDA (nmol/mgprot)** |
| Control | 131.42 ± 16.24 | 6.76 ± 1.54 | 3.46 ± 1.11 |
| Model | 77.70 ± 7.63b | 2.55 ± 0.78b | 7.58 ± 3.10b |
| Ly 5mg/kg | 90.95 ± 9.56c | 2.68 ± 1.26 | 5.24 ± 1.46d |
| Ly 10mg/kg | 109.52 ± 11.34d | 3.76 ± 0.91c | 4.13 ± 1.13d |
| Ly 20mg/kg | 121.25 ± 10.68d | 4.79 ± 1.51d | 3.45 ± 1.39d |

Data are expressed as the mean ± SD (*n* = 12) in each group. b*p* < 0.01 *vs* control group;c*p* < 0.05, d*p* < 0.01 *vs* model group. Ly: Lycopene; SOD: superoxide dismutase; GSH: glutathione; MDA: malondialdehyde.

**Table 3** **Effect of lycopene on low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and free fatty acid levels in liver tissue**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **LDL-C (mmol/L)** | **HDL-C (mmol/L)** | **FFA (mmol/L)** |
| Control | 0.34 ± 0.08 | 0.98 ± 0.10 | 0.82 ± 0.13 |
| Model | 2.48 ± 0.13b | 0.55 ± 0.02b | 2.03 ± 0.15b |
| Ly 5mg/kg | 1.32 ± 0.10c | 0.70 ± 0.04c | 1.73 ± 0.12 |
| Ly 10mg/kg | 0.95 ± 0.05d | 0.80 ± 0.05d | 1.56 ± 0.10c |
| Ly 20mg/kg | 0.62 ± 0.08d | 0.87 ± 0.05d | 1.34 ± 0.08d |

Data are expressed as the mean ± SD (*n* = 12) in each group. b*p* < 0.01 *vs* control group;c*p* < 0.05, d*p* < 0.01 *vs* model group. Ly: Lycopene; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; FFA: free fatty acid.