ANTI NECRO-INFLAMMATORY EFFECT OF STANDARDIZED PUNICA GRANATUM EXTRACT (40% ELLAGIC ACID) ON LIVER FIBROSIS INDUCED BY BILE DUCT LIGATION IN RATS

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ABSTRACT

The aim of this study was to evaluate the anti necro-inflammatory effect on liver fibrosis treated by standardized punica granatum extract. A total of thirty two albino rats (Rattus Norwegicus), male, Wistar strain were divided into 4 groups namely sham operated group (group I) and three main bile duct ligation groups (BDL) (group II = BDL, non-treated; group III = BDL, treated by 60 mg/kg body weight/day ellagic acid and group IV = BDL, treated by 150 mg/kg body weight/day standardized punica granatum extract respectively). The results showed a significative decrease of the necro-inflammatory score and fibrosis score for group III and group IV compared to group II but higher than group I ($p < 0.05$). The necroinflammatory value were higher in the group III compared to others group ($p < 0.05$). It can be concluded that the administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and avoided progression of fibrosis by its anti necro-inflammatory activity.

Keywords: anti necroinflammatory, liver fibrosis, punica granatum extract, bile duct ligation (BDL), hepatoprotective

INTRODUCTION

Liver fibrosis is the excessive accumulation of extracellular matrix proteins (ECM) in the liver (Bataller and Brenner, 2005). Patients with liver fibrosis can be asymptomatic for 15-20 years with morbidity and mortality only occurring after progression to cirrhosis (Friedman, 2003; Bataller and Brenner, 2005). Liver fibrosis was historically thought to be a passive and irreversible process due to the collapse of the hepatic parenchyma and its substitution with a collagen-rich tissue (Albanis and Friedman, 2001).

Recently, there is growing interest in understanding the role and mechanism of the phytochemicals: polyphenolics, flavonoids and phenyl propanoids as inhibitors of oxidative stress. Among all phytochemicals, ellagic acid (EA) has been receiving the most attention because of its wide array of biological properties, such as radical scavenging, chemopreventive, antiviral and antibacterial properties. It is mostly abundant in berries, walnuts, pecans, pomegranate, cranberries and other plant foods in the forms of hydrolysable tannins called ellagitannins (Devipriya *et al*., 2007). The aim of this study was to evaluate the degree of necroinflammatory activity on liver fibrosis treated by standardized punica granatum extract in order to find new otentical sources of hepatoprotective and antifibrotic agent.

MATERIALS AND METHODS

Chemicals used

Standardized punica granatum extrats (40% ellagic acid) and ellagic acid 90% were obtained from Xi'an Biof Bio-Technology Co., Ltd. (Room 1-1111, High-tech Venture Park, No. 69 Jinye Road, Gaoxin Distric of Xi'an, People Republic of China). Standardized punica granatum extract was administered at a dose level of 150 mg/kg body weight/day and ellagic acid 60 mg/kg body weight/day.

Experimental design

Male Wistar albino rats (200–250 g) were housed in a room at a mean constant temperature of 37° C with a 12 hour light-dark cycle, and free access to standard pellet chow and water. Biliary cirrhosis was induced surgically through double ligation and division of the common bile duct under combination of ketamine H Cl and diazepam (100 mg/ml: 5 mg/ml) at dose of 1 ml/kg body weight.

After 2 days from surgery 32 animals were divided into 4 groups (each group contain of 8 rats); sham operated group (group I) and three main bile duct ligated groups (BDL) (group II = BDL, non-treated; group III = BDL, treated by 60 mg/kg body weight/day ellagic acid and group IV = BDL, treated by 150 mg/kg body weight/day standardized punica granatum extract respectively). All these treated groups were treated for 3 weeks.

Histological studies

Small liver slices were rapidly removed, fixed in buffer formalin 10% fluid and stained with hematoxylin and eosin for histological examination underlight microscopy. For analysis, ten power fields per each liver section were done at X 10 and 40 (10 and 40 objective X 10 ocular). Necro-inflammatory scoring was taken by algorithm for ecro-inflammatory ctivity evaluation in Metavir System (Brunt, 2000). The degree of necro-inflammatory and fibrosis staging has been analysed by using the Kruskal–Wallis one-way analysis of variance.

RESULTS AND DISCUSSION

As shown in Table 1 necro-inflammation score was significantly higher in BDL rat treated with EA compared to the other groups ($p < 0.050$). While punica granatum extract administration in BDL(group IV) reduced this values significantly near of group I ($p < 0.05$). In BDL rat, score of fibrosis significantly increased compared to group I, III and IV ($p < 0.05$), while there was no significant difference between group III and IV (Table 1).

Table 1. The Average of Necro-inflammatory and Fibrosis Scores

Group	Necro-Inflammatory Score (Mean ±SD)	Fibrosis Score (Mean ± SD)
I (Sham Operation)	$0.375^{a} \pm 0.510$	$1.000^{a} \pm 0.000$
II. (BDL)	$0.875^{b} \pm 0.350$	$2.625^{c} \pm 0.518$
III (BDL + EA)	$1.250^{c} \pm 0.460$	$2.250^{b} \pm 0.463$
IV (BDL + PG)	$0{,}500^{a} \pm 0{,}530$	$1.875^{b} \pm 0.667$

Note: BDL= Bile Duct Ligation; EA =Ellagic Acid; PG= Promegranate Extract
The different superscript in the same column is significantly different ($p< 0.05$)

In sham operated groups, no histological alterations were detected (Fig.1A). On the other hand, all examined liver sections from BDL group show progression of fibrosis after 3 weeks. The hepatic architecture was completely distorted, liver lobules nearly disappeared and were replaced with wide fibrous tissue area admixed with highly proliferated and dilated bile ductules. Hepatic cells appeared with variable changes in their nuclei including hypertrophy, marginal chromatin, pyknosis, clumped, crescent shape, and irregular nuclear envelope. Mild to moderate inflammation and prominent enlarged Kupffer cells were seen in the fibrous tissue stroma (Fig 1B).

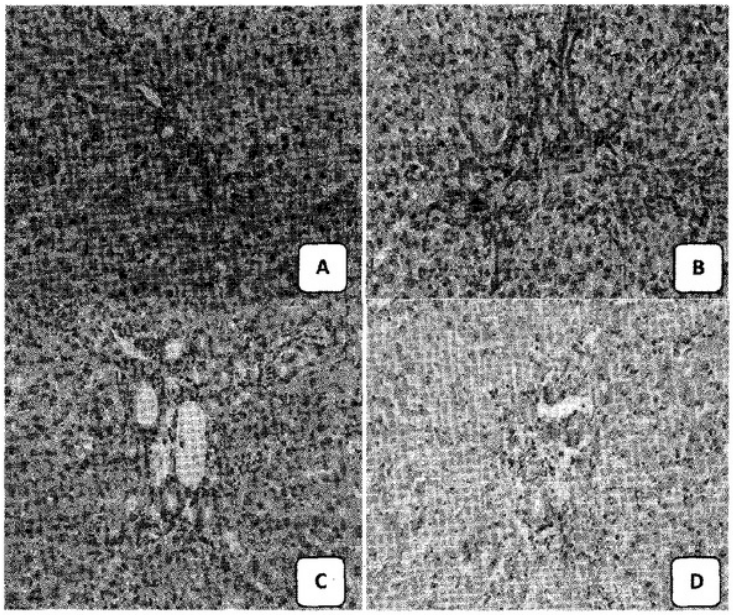

Figure 1. Liver section of: A: sham operated rat; B: Liver section of common bile duct ligated rat for 3 weeks; C: Section of liver of BDL rat treated with 60 mg ellagic acid/kg for 3 weeks and D: Section of liver of BDL rat treated with 150 mg standardized punica granatum extract/kg for 3 weeks (HE, X 100).

In BDL groups treated daily with 60 mg ellagic acid/kg for 3 weeks, the histological of liver sections still showing evidence of marked loss of hepatic lobular architecture. However, lobular architecture started to be distinguished, still show well organized hepatic lobular architecture with appearance of inflammatory cells and marked reduction in number of the proliferated bile ductules (Fig 1C).

Our study has demonstrated that oral administration of EA (60 mg/kg body weight) effectively decreased the degree of fibrosis but the necroinflammation degree of the liver still higher, so EA inhibit the progression of liver fibrosis by prolong the inflammatory process. This can be attributed to the antioxidant and the antiinflammation effect of EA.

Ellagic acid, a as phenolic compound can act as scavenging free radicals (Gil *et al.*, 2000), an antioxidant and inhibits cell injury (Priyadarsini *et al.*, 2002; Jung *et al.*, 2010). Furthermore, it has been reported that EA decreases the liver marker enzymes during CCl4-induced toxicity (Thresiamma *et al.*, 1996). Hence, it could be suggested that hepatocellular injury is decreased by the antioxidant action of EA. Since ROS have been implicated in the development of various pathological conditions, EA has the ability to control these diseases through its potential antioxidant activity.

The antiinflammatory effects of ellagic acid have been demonstrated in various animal model studies. In addition, the anti-inflamatory activity of EA was also investigated by using a standardized pomegranate rind extract containing 13% EA and finally proved to possess anti-inflammatory activity (Panichayupakaranant *et al.*, 2010).

Examination of BDL rats treated with 150 mg standardized punica granatum extract/kg/day for 3 weeks revealed moderate improvement in the hepatic lobular architecture. Well formed hepatocellular lobulation with slightly dilated sinusoids was seen in all examined slide. The hepatic lobules were separated with only thin bands of fibrous tissue, infiltrated with moderately dense inflammatory

cells. Few bile ductules were occasionally seen in portal areas and most of the hepatocytes appeared with normal nuclei (Fig 1D).

Inflammation, the first physiological defense system in the human body, can protect against injuries caused by physical wounds, poisons, and so on. However, long-term over-inflammation might cause such dysfunctions of the regular physiology as asthma, liver cirrhosis and rheumatic arthritis (Lee *et al.*, 2010). Many studies have pointed to the antiinflammatory properties of pomegranate fruit (Lansky and Newman 2007; Shukla *et al.*, 2008; Larrosa *et al.*, 2010; Lee *et al.*, 2010).

The administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and inhibit progression of fibrosis by decreasing the necro-inflammatory activity. It is suggest that antioxidant and antiinflammatory activity of the punica granatum extract are better than EA. The components of pomegranate extract that containt of EA and another compound might appear to synergistically suppress inflammatory cytokine expression. More recently, a whole pomegranate methanol extract was also shown to inhibit, in a dose-dependent manner, the production and expression of TNFα in microglial cells (Jung *et al.*, 2006), IL-1-induced expression of matrix metalloproteinases in human chondrocytes *in vitro* (Ahmed *et al.*, 2005), the expression of vascular inflammation markers, thrombospondin (TSP), transforming growth factor-β1 (DeNigris *et al.*, 2007) and inhibited cytokine IL-8, prostaglandin PGE2, and nitric oxide secretion, due to the action of the EA that also present in pomegranate (Romier-Crouzet *et al.*, 2009).

The antioxidant activity of pomegranate components due to the diverse phenolic compounds present in pomegranate. These compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation *in vitro* (Gil *et al.*, 2000). However, Tzulker *et al.*, (2007) suggested that punicalagin originating from the peels is one of the major phytochemicals contributing to the total antioxidant capacity of pomegranate juice. Madrigal-Carballo *et al.*, (2009) suggested that phenolic hydroxyl groups in punica granatum extract donate hydrogen to reduce free radicals. However, Amarowicz *et al.*, (2004) sugested that the antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals or chelate metal cations. Tzulker *et al.* (2007) reported that the homogenates preparation from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril juice.

CONCLUSION

It can be conclude that the increase of the necro-inflammatory grade could accelerate fibrosis progression and the administration of 150 mg/kg body weight/day standardized punica granatum extract possessed hepatoprotective activity and inhibit fibrosis progression by decreasing the necro-inflammatory activity.

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