Hepatoprotective Potential of Lycopene on D-Galactosamine/Lipopolysaccharide Induced Hepatitis in Rats

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Summary

Lycopene, a nutritional antioxidant was evaluated for its hepatoprotective potential against D-Galactosamine/Lipopolysaccharide (D-GalN/LPS) induced hepatitis in rats. Rats were given a single intraperitoneal injection of D-GalN/LPS (300 mg/kg body weight and 30 μg/kg body weight) to induce liver damage. Lycopene was administered to rats (10 mg/kg body weight for 6 days) 18 h before D-GalN/LPS challenge. D-GalN/LPS intoxication resulted in liver injury as indicated by the significant increase (p < 0.05) in the serum activities of marker enzymes such as aspartate amino transferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and γ-glutamyl transpeptidase. Further, there was a significant increase (p ≤ 0.05) in the levels of cholesterol, triglycerides and free fatty acids followed by a decrease in the levels of phospholipids in serum and liver. Pretreatment with lycopene reversed these alterations to near normal. Results of this study revealed that lycopene could afford a significant protection in the alleviation of D-GalN/LPS induced hepatocellular injury.

Keywords: lycopene, D-galactosamine, lipopolysaccharide, hepatitis, marker enzymes, lipids.
Introduction

Acute hepatitis can have serious health effects including mortality. Common causes of acute hepatitis include viral infection, side effects of certain prescription drugs and overdoses of the over-counter drugs [1]. Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents search for newer drugs continues because the existing synthetic drugs have several limitations. Hence crude drugs or natural food diet which possess antioxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health [2].

Lycopene has attracted much attention for its important roles in human health in particular as a dietary antioxidant with free radical scavenging activity [3]. Lycopene is a major carotenoid, available primarily from tomatoes and its products. Of all carotenoids, lycopene has been shown to exhibit the highest physical quenching rate constant with singlet oxygen [4]. Lycopene is known to exert protective effects against carcinogen-induced lung, liver and mammary tumours in experimental animals [5] and various diseases [6].

Among the numerous models of experimental hepatitis, D-GalN induced liver damage is very similar to human viral hepatis in its morphological and functional features [7]. D-GalN given at the time of endotoxin challenge sensitizes mice and other species to the lethal effects of endotoxin [8]. This immunological liver injury model has been used to evaluate the efficacy of several hepatoprotective agents [9].
Administration of antioxidants prior to D-GalN/LPS treatment has been used as a model to test the potential preventive role of natural phytochemicals. Biochemical studies on the effect of lycopene against D-GalN/LPS induced hepatitis have not been reported. Hence this study was designed to evaluate the therapeutic efficacy of lycopene against D-GalN/LPS induced changes in marker enzymes and lipid metabolism.

**Materials and Methods**

**Chemicals**

D-GalN and LPS (Sero type 011.B4 extracted by phenol water method from *E. Coli*) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade. Jagsonpal Pharmaceuticals, New Delhi, India, kindly provided Lycopene.

**Lycopene stock solution**

Lycopene (100 mg) was mixed in 2 ml Tween-80 at room temperature until a homogeneous paste was obtained. Physiologic saline at room temperature was added, drop wise and with vigorous stirring, to a final concentration of 10 mg lycopene/ml of suspension [10].

**Experimental animals**

Male Wistar rats of body weight 120-140 g obtained from Tamilnadu University Veterinary and Animal Sciences, Madhavaram, Chennai were used for this study. They were acclimatized to animal house conditions and were fed commercial pelleted diet.
(Hindustan Lever Limited, Bangalore, India) and water ad libitum. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

The animals were divided into 4 groups of six animals each. Group 1 served as vehicle control and was administered with tween 80 in saline. Group 2 rats were given lycopene 10 mg/kg body weight for 6 days (i.p). Group 3 rats were induced with hepatitis by giving intraperitoneal injections of D-GalN and LPS (300 mg/kg body weight and 30 µg/kg body weight) 18 hrs before the experiment [11]. Group 4 rats were pretreated with lycopene for 6 days prior to the induction of D-GalN/LPS. The rats were anesthetised and sacrificed after the experimental period by cervical decapitation. Blood was collected and the serum separated was used for biochemical analysis. The liver tissue excised was washed with ice-cold saline. A portion of the liver was then homogenized in 0.1 M Tris buffer and the homogenate was used for biochemical estimations.

**Collection of samples for biochemical and histological analysis**

After the experimental period, the animals were anaesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight) and sacrificed. Blood was collected and the liver tissue was excised quickly. The tissues were washed in physiological saline to remove blood clot and other tissue materials. For histopathological studies, a piece, approximately of 1 cm$^3$ of liver tissue was cut and placed immediately in phosphate buffered formal saline (pH 7.4).
Biological assays

The activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel [12] and the levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined by the methods of King and Armstrong [13]; King [14] respectively. Gamma glutamyl transferase (γ-GT) activity was assayed by Rosalni and Rau method [15].

The extraction of serum and tissue lipids was done according to the procedure of Folch et al, [16]. The estimation of total cholesterol was carried out by the method of Zlatkis et al., [17] and triglycerides by the method of Foster and Dunn Foster et al. [18]. Free fatty acids was estimated by the method of Falholt et al. [19] and phospholipids by the method of Zilversmit and Davis, [20].

Statistical analysis

All the grouped data were statistically evaluated with SPSS 7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference test. P value of less than 0.05 was considered to indicate statistical significance. All the results were expressed as mean ± SD for six animals in each group.
Results

In d-GalN/LPS intoxicated rats, the activities of AST, ALT, ALP, LDH and γ-GT in serum were increased significantly when compared with control (Table 1). In contrast, pretreatment with lycopene decreased significantly these elevated parameters when compared with d-GalN/LPS induced group.

Table 1. Levels of Serum AST, ALT, ALP, LDH and γ-GT in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>ALP (IU/l)</th>
<th>LDH (IU/l)</th>
<th>γ-GT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>102.57 ± 9.50</td>
<td>48.61 ± 3.80</td>
<td>45.90 ± 4.81</td>
<td>102.20 ± 7.83</td>
<td>6.85 ± 0.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>103.29 ± 9.32</td>
<td>50.02 ± 4.01</td>
<td>48.12 ± 5.1</td>
<td>101.00 ± 8.71</td>
<td>7.05 ± 0.8</td>
</tr>
<tr>
<td>Group 3</td>
<td>369.84 ± 26.19(^a)</td>
<td>170.34 ± 15.73(^a)</td>
<td>139.00 ± 11.85(^a)</td>
<td>191.00 ± 13.94(^a)</td>
<td>15.15 ± 3.00(^a)</td>
</tr>
<tr>
<td>Group 4</td>
<td>141.73 ± 8.54(^b)</td>
<td>63.51 ± 4.45(^b)</td>
<td>58.17 ± 6.18(^b)</td>
<td>111.00 ± 8.75(^b)</td>
<td>7.32 ± 0.9(^b)</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each.
Values are statistically significant at \(^p<0.05\)
Comparison are made as \(^\text{a} Group 1\) and \(^\text{b} Group 3\).

The levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum and liver respectively are shown in Table 2 and Table 3. The levels of cholesterol, triglycerides and free fatty acids were significantly increased while that of phospholipids was decreased in rats injected with d-GalN/LPS. These altered levels were brought back to near normal when pretreated with lycopene.
Table 2. Levels of Serum cholesterol, triglycerides, free fatty acids and phospholipids in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Free fatty acids (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>75.64 ± 7.1</td>
<td>47.22 ± 4.1</td>
<td>19.32 ± 1.24</td>
<td>107.40 ± 7.92</td>
</tr>
<tr>
<td>Group 2</td>
<td>78.11 ± 6.92</td>
<td>47.38 ± 3.9</td>
<td>20.16 ± 1.97</td>
<td>109.81 ± 9.1</td>
</tr>
<tr>
<td>Group 3</td>
<td>104.62 ± 9.1a</td>
<td>111.21 ± 9.42a</td>
<td>35.04 ± 2.73a</td>
<td>75.21 ± 6.1a</td>
</tr>
<tr>
<td>Group 4</td>
<td>81.04 ± 7.1b</td>
<td>58.13 ± 4.93b</td>
<td>25.37 ± 2.18b</td>
<td>98.40 ± 8.53b</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *p<0.05. Comparison are made as a Group 1 and b Group 3.

Table 3. Levels of cholesterol, triglycerides, free fatty acids and phospholipids in liver of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Free fatty acids (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>11.20 ± 0.96</td>
<td>23.80 ± 1.28</td>
<td>0.70 ± 0.04</td>
<td>25.30 ± 1.70</td>
</tr>
<tr>
<td>Group 2</td>
<td>11.62 ± 0.88</td>
<td>24.07 ± 1.79</td>
<td>0.71 ± 0.03</td>
<td>24.68 ± 2.19</td>
</tr>
<tr>
<td>Group 3</td>
<td>19.58 ± 1.20a</td>
<td>42.14 ± 1.69a</td>
<td>2.20 ± 0.10a</td>
<td>16.94 ± 1.43a</td>
</tr>
<tr>
<td>Group 4</td>
<td>14.00 ± 0.89b</td>
<td>30.01 ± 2.56b</td>
<td>1.27 ± 0.09b</td>
<td>21.47 ± 1.79b</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *p<0.05. Comparison are made as a Group 1 and b Group 3.

Figure 1 reveals the histopathological observation of the liver tissue in control and experimental groups of rats. (Figure.1a and Figure.1b) show liver sections of control (Group 1) and Lycopene alone treated (Group 2) rats with normal architecture. GalN/LPS induction (Group 3) produced centrilobular necrosis with loss of architecture (Figure.1c).
When rats were pretreated with Lycopene (Group 4), no hepatic necrosis was seen (Figure 1d). Appreciable adverse side effects were not observed in rats treated with lycopene alone. Lycopene causes no abnormal observable metabolic disturbance of the system and there were no toxic or deleterious effects.

**Fig 1:** Histopathological examination of liver sections in control and experimental rats (Haematoxylin and eosin stain 10 x 10X).

**Figure. 1a.** Illustrates a section of control rat liver showing normal architecture.

**Figure. 1b.** Illustrates a section of liver of Lycopene treated rat showing normal Liver parenchyma with central vein and cords of hepatocytes.

**Figure. 1c.** Illustrates a section of D-GalN/LPS induced rat liver which shows loss of architecture and cell necrosis (perivenular) extending to the central zone. The cell necrosis with inflammatory collections is more prominent in the central zone than around central vein.

**Figure. 1d.** Illustrates a section of rat liver pretreated with lycopene prior to D-GalN/LPS Challenge showing central vein surrounded by hepatocytes with sinusoidal Dilatation with occasional inflammatory cells. No hepatic necrosis was seen around central vein in the central zone.
Discussion

D-GalN/LPS induced hepatocellular damage, a well-established model of hepatitis takes advantage of the ability of D-GalN to potentiate the toxic effects of LPS producing fulminant hepatitis within a few hours of administration [21]. A high dose of D-GalN causes necrosis of the liver by UTP depletion and inhibition of protein synthesis, although D-GalN is often used in combination with lipopolysaccharide or tumour necrosis factor [22]. Accumulation of UDP-sugar nucleotides [23; 24] may contribute to the changes in the rough endoplasmic reticulum and to the disturbance in the protein metabolism. Further, intense galactosamination of membrane structure is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death [25]. In recent years, apart from the well documented inhibition of protein synthesis, it has been suggested that reactive oxygen species produced by activated macrophages might be the primary cause in D-GalN-induced liver damage [26; 27].

Liver damage induced by D-GalN/LPS generally reflects disturbances of liver cell metabolism which lead to characteristic changes in the activities of serum enzymes. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membranes [28]. In this context, we have also observed a significant increase in the serum activities of AST, ALT, ALP, LDH and \( \gamma \)-GT which is in accordance with the earlier findings [24; 29]. Because the levels of these marker enzymes are proportional to the extent of damage, the activity of these enzymes can be used for diagnosis as indicators of prognosis of the disease.
The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage [30]. Lycopene seems to preserve the structural integrity of the hepatocellular membrane as evident from the significant reduction in these enzymes in rats induced with hepatitis.

ALP is a membrane bound glycoprotein enzyme and has been shown using histochemical techniques to be present in high concentration in the sinusoids and the endothelium of the central and periportal veins [28]. It has been reported to be involved in the transport of metabolites across the cell membranes, protein synthesis, synthesis of certain enzymes, secretory activities and glycogen metabolism. Thus the rise in serum ALP activity in rats induced with hepatitis may be due to a disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes as in the other hepatotoxic conditions. Sharma et al. 1995. Pretreatment with lycopene brought back the enzyme level to near normal indicating clearly the therapeutic value of lycopene.

LDH, a sensitive indicator of liver injury was also raised in serum in D-GalN/LPS induced rats. This increased activity of LDH in serum confirms the increased permeability of the hepatocyte membrane and cellular leakage Paduraru et al. [32]; Premalatha et al, [33] have also reported that the increase in LDH may be due to the release of isoenzyme from destroyed tissue [33].These levels were reversed back to near normal in lycopene pretreated rats.

γ-GT is most sensitive indicator of liver disease and is a useful marker in patients with liver metastases. A number of drugs and chemicals are known to increase γ-GT activity.
by the induction of microsomal enzyme [34]. In the present observation, the activity of γ-GT showed significant increase due to D-GalN/LPS induction. This may be due to the fact that the depletion of GSH may induce hepatic γ-GT activity through an increased synthesis of its mRNA [35]. Pretreatment with lycopene in liver damaged rats showed reduction in γ-GT activity, thus revealing the membrane stabilising activity of lycopene. This implies that supplementation of lycopene is indicative of the improvement of the liver.

The reversal of increased serum enzyme activities in D-GalN/LPS induced hepatic damage may be due to the healing of parenchyma and the regeneration of hepatocytes by its membrane stabilising activity. This was well correlated with the protective action of natural antioxidants (Cianidanol and Silymarin) and synthetic antioxidant (N-acetylcystein) which showed changes in marker enzymes against liver injury induced by D-GalN in rats [36].

The liver is the major site of cholesterol, bile acids and phospholipid synthesis and metabolism [37]. Marked alterations in lipid metabolism have been reported in D-GalN induced hepatitis in rats [38]. Our results also showed increased levels of plasma and tissue cholesterol, triglycerides, free fatty acids with a decrease in phospholipids in D-GalN/LPS challenged rats when compared to control.

Hepatic cholesterol homeostasis is maintained by an equilibrium between the activities of hydroxy methyl glutaryl CoA (HMG-CoA) reductase and that of acyl CoA : Cholesterol acyl transferase activity on the other hand [37]. The increased cholesterol level may be due to increased HMG-CoA reductase activity which is the rate limiting step in cholesterol biosynthesis [39]. This finding could be correlated with the results of the previous reports [40; 41].
The increase in cholesterol increases the membrane fluidity, regulates membrane permeability and alters internal viscosity and also the internal chemical composition [42]. Fatty acids are the principle components present in most lipids of biological importance. The increase in the levels of free fatty acids is due to the fact that increased peroxidation of the membrane phospholipids releases free fatty acids by the action of phospholipase A₂ [43]. Ca²⁺ ions have been reported to be one of the inducers of phospholipase A₂. So the increased levels of free fatty acids in D-GalN/LPS intoxicated group might be due to the indirect effect of calcium level which was reported to be elevated upon d-GalN administration [25].

Liver injury causes the accumulation of abnormal amounts of fats, predominantly triglycerides in the parenchymal cells. Triglyceride accumulation can be thought of as resulting from an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into the systemic circulation [44]. The elevated plasma triglyceride levels observed might have been partially due to lipoprotein lipase.

In free radical mediated tissue injury, lipid peroxidation leads to degradation of phospholipids and alteration in the membrane fluidity which is essential for liver cell function [45]. The decreased level of phospholipids observed is due to increased activity of phospholipase A and phospholipase C [28].

Rats pretreated with lycopene prior to the induction of hepatic damage showed a restoration of the altered lipid levels induced by d-GalN/LPS towards near normalcy thereby showing the modulating effect of lycopene against d-GalN/LPS induced changes in lipid levels in rats.
The histological evidence authenticated the injury caused by D-GalN/LPS and the protection offered by Lycopene to hepatocytes. Microscopical examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in D-GalN/LPS - induced rats. Prior oral administration with Lycopene extract prevented completely the histopathological changes in liver induced by D-GalN/LPS. Liver sections from rats treated alone with Lycopene showed normal morphology without appreciable histological abnormalities. Thus the histopathological studies serve as a direct evidence of efficacy of drug as protectant. The results of histopathological study also support the result of biochemical parameters and explain the hepatoprotective activity of Lycopene.

Thus the present study confirms the hepatoprotective action of lycopene against D-GalN/LPS induced hepatitis in rats. The protective action of lycopene against D-GalN toxicity is of clinical importance because there is close resemblance between the multifocal necrosis produced by D-GalN and the lesion of viral hepatitis in humans. The results of this study suggest that lycopene could serve as a better remedy for liver disease and controlled clinical studies in viral hepatitis would be worthwhile.

Acknowledgement

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