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Lycopene stabilizes liver function during D-galactosamine/lipopolysaccharide induced hepatitis in rats

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Abstract

Hepatitis remains as clinical challenge and a problem of great importance in developing and underdeveloped world. Acute hepatitis can have serious health effects including mortality. There is no specific treatment for acute hepatitis. Care is aimed at maintaining comfort and adequate nutritional balance. Lycopene is a potent antioxidant of carotenoid family found in fruits and vegetables. The consumption of lycopene-rich foods, such as tomato paste, chilli sauce, and spaghetti sauce, has been demonstrated to prevent the occurrence of a number of chronic diseases including various types of cancers. Lycopene has been associated with a number of health benefits particularly in regard to prostate, lung, heart and skin health. The present investigation was carried upon to explore the role of lycopene on liver health by analyzing the biochemical parameters and liver marker enzymes during experimentally induced hepatitis in animal model and the findings strongly suggest that lycopene is potential agent of hepatoprotection.

Keywords: Lycopene; Antioxidant; Hepatitis; Galactosamine; Free radical

1. Introduction

The viral hepatitis particularly, Hepatitis B is the most common form of acute hepatitis. Hepatitis B virus (HBV) is a major cause of acute hepatitis, cirrhosis and hepatocellular carcinoma worldwide. HBV continues to be the single most important cause of viral hepatitis in the developing and underdeveloped world. It is estimated that about one-third of the global population, around 2 billion people have been infected with the hepatitis B virus at some stage in their lifetime. Of these, about 360 million people remain chronically infected carriers of the disease. According to World Health Organisation (WHO) report [62], an estimated 600,000 persons die each year due to the acute or chronic consequences of hepatitis B. Hepatitis remains as clinical challenge and a problem of great importance. Acute hepatitis can have serious health effects including mortality. There is no specific treatment for acute hepatitis. Care is aimed at maintaining comfort and adequate nutritional balance [22].

Carotenoids are a class of more than 600 natural pigments that are present in fruits and vegetables [23]. Epidemiological reports demonstrate a clear inverse association between diets high in carotenoid-rich fruits and vegetables and reduced incidence of variety of diseases [59,60]. Lycopene is a potent antioxidant of carotenoid family, and naturally occurring compound that gives characteristic red color to tomato, watermelon, pink grapefruit, orange, and apricot [51]. A number of studies have indicated the health benefits of consuming lycopene and demonstrated its preventive role in

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the occurrence of a number of chronic diseases including various types of cancers [17,24,16,12,1,11,57]. Lycopene is found to be the most effective antioxidant among all the tested carotenes and xanthophylls [36,13]. Among the numerous models of experimental hepatitis, D-GalN induced liver damage is very similar to human viral hepatitis in its morphological and functional features [31]. Administration of a subtoxic dose of galactosamine (GalN) together with or followed by lipopolysaccharide (LPS) induces acute hepatitis and is a well-established model for acute hepatitis [4,63,41]. This liver injury model has been used to evaluate the therapeutic value of flavones, quinines and carotenoids that are known as antioxidants and of other plant products that are claimed to be hepatoprotective [39,19,29].

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 possesses 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. The scientific research to date has demonstrated an array of health benefits clearly associated with lycopene. It offers important health benefits particularly in regard to prostate, lung, heart and skin health. In our preliminary investigation we have earlier reported the role of lycopene on liver health in experimental animals [52,49,50]. Thus the present investigation further explores the hepatoprotective role of lycopene by analyzing the biochemical parameters and liver marker enzymes during D-GalN/LPS induced liver injury.

2. Materials and methods

2.1. Chemicals

D-GalN and LPS (Sero type 011.B4 extracted by phenol water method from *Escherichia coli*) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals (acids, bases, solvents and salts) used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratoreis, CDH division, Mumbai, India. Jagsonpal Pharmaceuticals, New Delhi, India, kindly provided Lycopene.

2.1.1. 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 [35].

2.2. Animals

Adult male albino rats of Wistar strain weighing around 120–150 g obtained from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, India were used in this study. They were housed in polypropylene cages over husk bedding and a 12 h light and dark cycle was maintained throughout the experimental period. Rats were fed a 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 (IAEC No. 01/026/08).

2.3. Experimental design

The animals were divided into four 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 alone (10 mg/kg body weight for 6 days intraperitoneally).
Group 3: Rats were induced with D-GalN and LPS (300 mg/kg body weight and 30 μg/kg body weight, i.p 18 h before the experiment) [42].
Group 4: Rats were pretreated with lycopene for 6 days prior to the induction of D-GalN/LPS.

2.4. Collection of samples for biochemical 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 immediately washed in physiological saline to remove blood clot and other tissue materials and stored at 4 °C until further use.

2.5. Separation of serum

The blood samples collected in plain centrifuge tubes were kept in inclined position to allow complete clotting of blood and then centrifuged at 2500 rpm for 30 min. The resultant clear supernatant was pipetted

out and preserved in small vials in the freezer for the purpose of biochemical investigations.

2.6. Preparation of liver homogenate

Within 3 h after sacrifice, liver samples were blotted to dryness. From this, a piece weighing about 100 mg was taken and homogenized at 4 °C in Tris–HCl buffer (0.1 M, pH 7.4). The tissue homogenates were centrifuged at 2500 rpm for 30 min. The resultant supernatant was kept under refrigeration until further biochemical analysis. All the assay procedures were carried out within 48 h after sample collection.

2.7. Estimation of urea, ammonia, uric acid, creatinine and bilirubin

The blood urea and ammonia were determined by the methods of Natelson et al. [41] and Kingsley and Tager [28] respectively. Their values were expressed as mg/dl in blood. Uric acid, creatinine and bilirubin were estimated in the serum according to the method of Caraway [10], Broad and Sirota [9] and Malloy and Evelyn [33] respectively. The values were expressed as mg/dl of serum.

2.8. Estimation of glucose, glycogen, lactate and pyruvate

Blood glucose was estimated by the method of Sasaki and Matsui [48]. The values were expressed as mg/dl blood. Liver glycogen was estimated by the method of Morales et al., [38]. The values were expressed as mg/g tissue. The lactate and pyruvate content of liver was estimated by the methods of Barker and Summerson [7] and Friedemann and Haugen [15] respectively. Their values were expressed as μg/mg protein.

2.9. Estimation of iron and ferritin

Iron content was estimated by the method of Ramsay [44]. The iron content of ferritin in liver was estimated by the method of Drysdale and Munro [14]. The results were expressed as μg/g wet liver.

2.10. Assay of diagnostic liver markers enzymes

The method of Reitman and Frankel [46] was adopted for the assay of asparate transaminase (AST) and alanine transaminase (ALT). These enzyme activities were expressed as μmoles of pyrvuate liberated/h/mg protein for tissue. Alkaline phosphatase was assayed by the method of Kind and King [25]. Acid phosphatase (ACP) was assayed by the method of King [26]. Their activities were expressed as μmoles of phenol liberated/h/mg protein for tissue. The assay of γ-glutamyl transferase (γGT) was carried out by the method of Rosalki and Rau [47]. Lactate dehydrogenase (LDH) was assayed according to the method of King [27]. The enzyme activity was expressed as μmoles of pyrvuate liberated/h/mg protein for tissue.

2.11. Histopathological studies

Small pieces of liver tissues were collected in 10% formal saline for proper fixation. These tissues were processed, embedded in paraffin wax sections of 5–6 μm thick, cut and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were obtained.

2.12. Statistical analysis

All the grouped data were statistically evaluated with Statistical Package for Social Sciences (SPSS), Version 10.0. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. A '*P*' value of less than 0.05 was considered to indicate statistical significance. All the results were expressed as mean ± S.D. for six animals in each group.

3. Results

3.1. Urea, ammonia, uric acid and creatinine

Figs. 1 and 2 depict the levels of urea, ammonia, uric acid and creatinine in control and experimental animals. Blood urea and serum uric acid levels were significantly

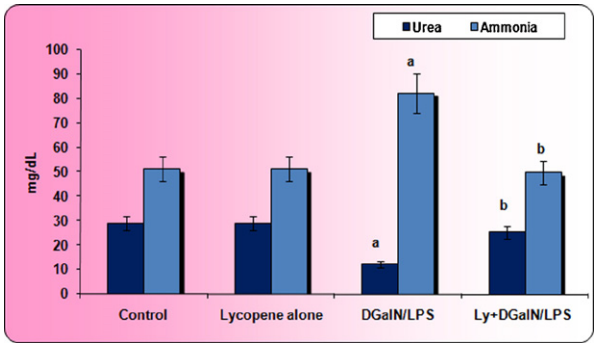

Fig. 1. Levels of urea and ammonia in control and experimental group of animals. Values are expressed as mean ± SD for six rats in each group. [a] As compared with group 1 (control), [b] As compared with group 3 (D-GalN/LPS); [a,b] represent $P < 0.05$. (Ly-lycopene).

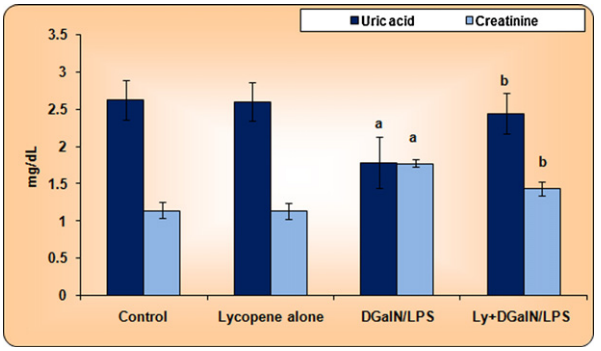

Fig. 2. Levels of uric acid and creatinine in control and experimental group of animals. Values are expressed as mean ± SD for six rats in each group. [a] As compared with group 1 (control), [b] As compared with group 3 (D-GalN/LPS); [a,b] represent $P < 0.05$. (Ly-lycopene).

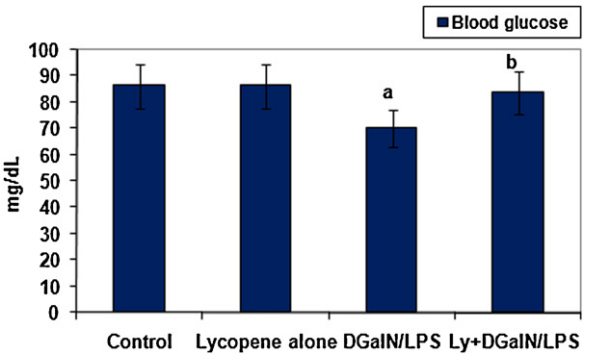

Fig. 3. Levels of glucose in control and experimental group of animals. Values are expressed as mean ± SD for six rats in each group. [a] As compared with group 1 (control), [b] As compared with group 3 (D-GalN/LPS); [a,b] represent $P < 0.05$. (Ly-lycopene).

decreased ($P < 0.05$) whereas blood ammonia and serum creatinine levels were significantly increased ($P < 0.05$) in rats induced with D-GalN/LPS (Group 3). The above changes were reversed to near normal levels in rats treated with lycopene prior to the toxic insult of D-GalN/LPS (Group 4). Group 2 rats treated with lycopene alone did not show any significant alteration in these parameters when compared to control (Group 1).

3.2. Bilirubin

Table 1 depicts serum levels of total, conjugated and unconjugated bilirubin in control and experimental groups of rats. Rats injected with D-GalN/LPS (Group 3) showed a significant elevation ($P < 0.05$) in total and conjugated bilirubin with subsequent reduction ($P < 0.05$) in unconjugated bilirubin when compared to control (Group 1). Pretreatment with lycopene (Group 4) resulted in reversal of above changes to near normal. Rats treated with lycopene alone (Group 2) did not show any significant changes in bilirubin levels when compared to control.

3.3. Glucose, glycogen, lactate and pyruvate

Figs. 3–5 represent the levels of glucose, glycogen, lactate and pyruvate in control and experimental groups

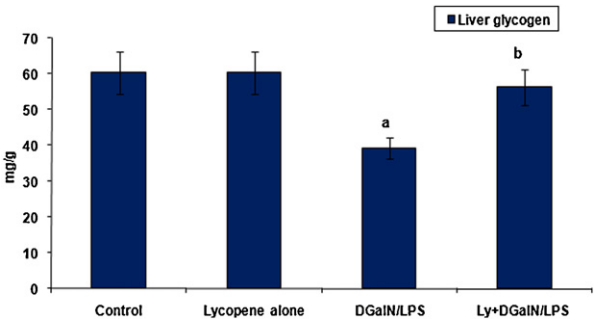

Fig. 4. Levels of glycogen in the liver of control and experimental group of animals. Values are expressed as mean ± SD for six rats in each group. [a] As compared with group 1 (control), [b] As compared with group 3 (D-GalN/LPS); [a,b] represent $P < 0.05$. (Ly- lycopene).

of rats. Diminished levels ($P < 0.05$) of blood glucose and glycogen were observed in addition to significantly elevated levels ($P < 0.05$) of lactate and pyruvate in liver upon D-GalN/LPS induction (Group 3) when compared to control (Group 1). Administration of lycopene (Group 4) before D-GalN/LPS injection brought back the above changes to near normal. Significant variations were not found in lycopene alone treated rats (Group 2) when compared to control.

Table 1
Levels of total, conjugated and unconjugated bilirubin in serum of control and experimental groups of rats.

Parameters	Group 1 Control	Group 2 Lycopene alone	Group 3 DGalN/LPS	Group 4 Lycopene + DGalN/LPS
Total bilirubin	0.45 ± 0.02	0.46 ± 0.02	0.85 ± 0.06[a]	0.58 ± 0.03[b]
Conjugated (direct) bilirubin	0.36 ± 0.03	0.35 ± 0.02	0.45 ± 0.02[a]	0.39 ± 0.02[b]
Unconjugated (indirect) bilirubin	0.09 ± 0.01	0.09 ± 0.01	0.40 ± 0.03[a]	0.19 ± 0.01[b]

Values are expressed as mean ± SD for six rats in each group. [a]As compared with group 1, [b]as compared with group 3; [a,b]$P < 0.05$.

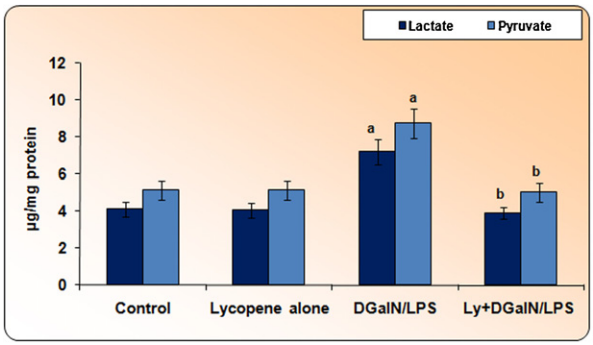

Fig. 5. Levels of lactate and pyruvate in control and experimental group of animals. Values are expressed as mean ± SD for six rats in each group. [a] As compared with group 1 (control), [b] As compared with group 3 (D-GalN/LPS); [a,b] represent $P < 0.05$. (Ly-lycopene).

3.4. Iron and ferritin

Table 2 shows the levels of iron and ferritin in control and experimental groups of rats. The level of iron and ferritin was significantly ($P < 0.05$) increased in D-GalN/LPS induced rats (Group 3) when compared with control rats (Group 1). Upon pretreatment with lycopene (Group 4), the increased levels of iron and ferritin were brought back to near normal. There was no change in the levels of these parameters in rats treated alone with lycopene (Group 2) when compared to control.

3.5. Marker enzymes

Table 3 represent the effect of lycopene on the activities of marker enzymes (AST, ALT, ALP, ACP, γGT and LDH) in liver of control and experimental groups of rats respectively. Abnormal increase ($P < 0.05$) in marker enzymes was observed in D-GalN/LPS induced rats (Group 3) when compared to control rats (Group 1). The enzymic activities were found to be at normal when pretreated with lycopene (Group 4). There seems to be no significant difference between the lycopene alone treated rats (Group 2) and control.

3.6. Hematoxylin and eosin staining

Histological analysis of liver section is illustrated in Fig. 6. Liver section of control and lycopene alone treated samples shows the normal architecture (a and b), where as DGalN/LPS induced (group3) liver section shows extensive cellular necrosis with loss of architecture (c). Lycopene pretreated group 4 sample shows the recovering cells and reduced necrosis (d).

4. Discussion

In hepatic injury, plasma urea declines significantly due to failure of the liver to convert amino acids and ammonia to urea. Several studies have reported decreased levels of urea during D-GalN-induced hepatitis in rats [2]. The results of our study coincide with the above findings. Urea production is related to metabolic pathways for disposal of ammonia, which is the toxic end product of nitrogen metabolism. Krebs et al., [30] have suggested that stoichiometric amounts of aspartate and carbamoyl phosphate are required for proper functioning of the urea cycle. Thus the reduction in the level of urea

Table 2
Levels of iron and ferritin in liver of control and experimental groups of rats.

Parameters	Group 1 Control	Group 2 Lycopene alone	Group 3 DGalN/LPS	Group 4 Lycopene + DGalN/LPS
Iron	149.13 ± 13.92	149.12 ± 13.92	323.22 ± 31.78[a]	175.53 ± 15.31[b]
Ferritin	37.88 ± 3.81	37.60 ± 3.75	54.06 ± 2.15[a]	39.42 ± 3.51[b]

Values are expressed as mean ± SD for six rats in each group. [a]As compared with group 1, [b]as compared with group 3; [a,b]$P < 0.05$.

Table 3
Activities of aspartate transminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in liver of control and experimental groups of rats.

Parameters	Group 1 Control	Group 2 Lycopene alone	Group 3 DGalN/LPS	Group 4 Lycopene + DGalN/LPS
AST	5.52 ± 0.48	5.53 ± 0.48	7.82 ± 0.69[a]	5.98 ± 0.54[b]
ALT	11.23 ± 1.12	11.15 ± 1.11	17.28 ± 1.55[a]	12.11 ± 1.22[b]
ALP	2.28 ± 0.24	2.24 ± 0.23	4.58 ± 0.42[a]	2.79 ± 0.31[b]
γ-GT	5.48 ± 0.52	5.46 ± 0.51	12.27 ± 1.21[a]	6.07 ± 0.59[b]
LDH	4.22 ± 0.41	4.20 ± 0.40	7.57 ± 0.69[a]	4.96 ± 0.51[b]

Values are expressed as mean ± SD for six rats in each group. [a]As compared with group 1, [b]as compared with group 3; [a,b]$P < 0.05$.

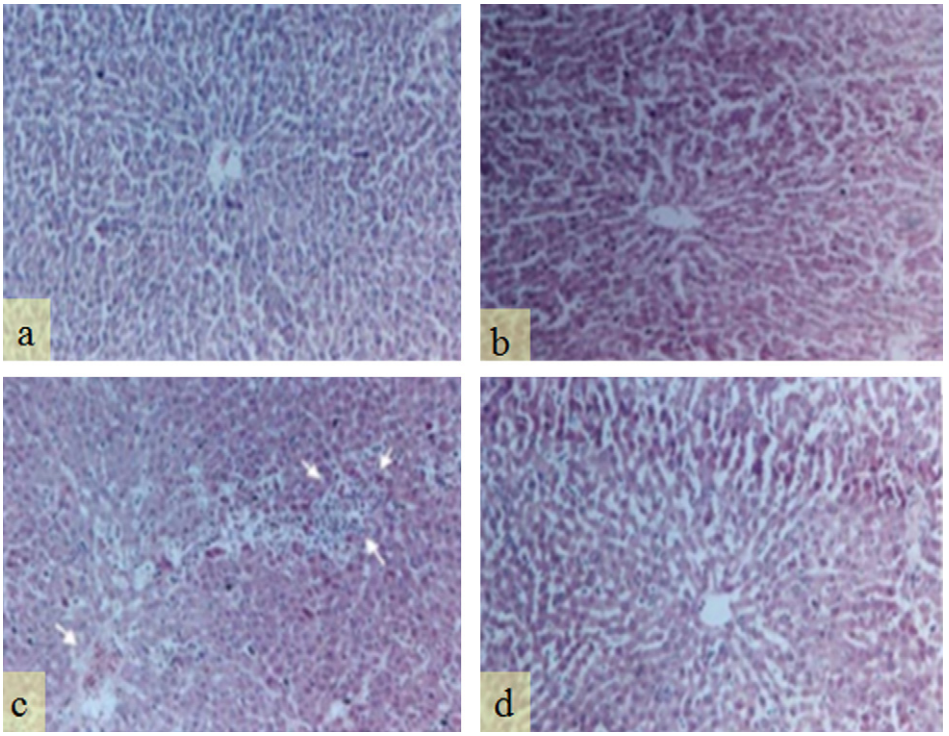

Fig. 6. Histopathological examination of liver sections in control and experimental group of animals (haematoxylin and eosin staining, 100x). (a). Illustrates a section of control rat showing normal architecture. (b). Illustrates a section of lycopene alone treated rat showing normal liver parenchyma with central vein and cords of hepatocytes. (c). Illustrates a section of D-Gal/LPS induced liver which shows loss of architecture and cell necrosis extending to the central zone. (d). Illustrates a section of rat liver pretreated with lycopene prior to D-Gal/LPS challenge showing central vein surrounded by hepatocytes with sinusoidal dilatation without hepatic necrosis.

may be due to the interference of D-GalN/LPS with the control mechanisms, which regulate the stoichiometric formation of these compounds. Alteration in the levels of ammonia and urea are potential markers of free radical damage to protein. There is an increased catabolism of proteins coupled with the diminished ability of kidneys to excrete nitrogenous waste. This could possibly account for the raised ammonia and lowered urea levels in the plasma of D-GalN-induced rats [53]. Uric acid, the metabolic end product of purine metabolism has been proven to be a selective antioxidant capable especially of reacting with free radicals and hydrochlorous acid [18]. Decreased levels of uric acid might be due to increased production of free radicals and subsequent lipid peroxidation. Creatinine is more readily excreted by kidney than urea or uric acid. Elevated levels of serum creatinine can be attributed to the increased breakdown of creatine phosphate for energy and conversion of creatine to creatinine for excretion. The restoration of the above parameters to near normal in lycopene pretreated group 4 rats could be due to the antioxidant defense of lycopene [6,56,5] that stabilizes the liver function and contributes to its anti-hepatotoxic potential [21,61].

Determination of serum bilirubin serves as an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function. The increased levels of unconjugated and conjugated bilirubin could result from an impairment of uptake or conjugation coupled with decreased excretion of the pigment. Increased levels of bilirubin in this study are in agreement with the reports of the previous studies that D-GalN-induced hepatitis is characterized by increased levels of bilirubin in serum [32,54]. The lycopene mediated suppression of the increased bilirubin level suggests the possibility of lycopene being able to stabilize biliary dysfunction.

The striking feature of D-GalN/LPS-induced hepatitis is a significant depletion of liver glycogen and hypoglycemia. Since liver contain a full complement of the necessary enzymes involved in glucose homeostasis, toxic drugs may alter the glucose level. Observation of significant decrease in blood glucose levels during D-GalN induction has been already reported [34]. Arai et al. [32], has suggested that, in D-GalN-induced liver failure model, hepatic glucose production is completely

arrested and the liver switches to a glycolytic mode even in a fasted animal. D-GalN causes the activation of pyruvate kinase and the glycolysis pathway. The switch from gluconeogenesis to glycolysis may be due to the depletion of ATP and probable accumulation of AMP in liver tissue as a result of fulminant hepatic failure. Hypoglycemia during liver injury is a consequence of impaired gluconeogenesis and inability to mobilize glycogen stores. In the present study the depleted glucose level in group 3 rats was appreciably improved in group 4 rats pretreated with lycopene suggesting that it may improve gluconeogenesis. The significant depletion in the levels of liver glycogen in rats given the hepatotoxin might be due to the rapid and extensive depletion of UTP, thereby not allowing the cycling of uridine nucleotides and consequently disrupting sugar and mucopolysaccharide metabolism. Thus the pathway leading to glycogen synthesis is diverted to glycolysis in injured liver since both pathways utilize glucose as the substrate. Our results suggest that group 4 rats pretreated with lycopene exhibited considerable prevention in the excessive depletion of liver glycogen. Administration of lycopene may protect the liver cells from injury [8] and stimulate them to convert more glucose to glycogen and hence glycogen content is restored to near normal levels. Pyruvate is the major physiological substrate for gluconeogenesis and hence impaired gluconeogenesis has been reported to result in the accumulation of lactate and pyruvate [45]. It was reported that with the induction of glycolysis in fulminant hepatic failure rat livers, there was no concomitant activation of pyruvate dehydrogenase to convert pyruvate to acetyl-CoA, which led to a significant increase in lactate release [3]. Pretreatment with lycopene prior to D-GalN/LPS induction showed a significant tendency for the maintenance of lactate and pyruvate in a near normal range. This may be brought about by a profound influence of lycopene on the vital enzymes of liver metabolism. These findings suggest the protective role of lycopene on cell membrane and on aberrant activities of carbohydrate metabolism.

Ferritin is valuable indirect marker of stored iron in liver parenchyma with different types of liver damages. Thus the evaluation of iron and ferritin levels is a valid tool during liver injury [20]. The increased level of ferritin and iron during hepatic injury was observed earlier [43,58,37] Our observation matches with the earlier findings and lycopene pretreated group 4 animals were able to restore the near normal values of iron and ferritin in comparison with group 1 control animals. Since iron is a reactive metal ion involved in damage to cellular macromolecules caused by oxygen radicals, its reduction from Fe^{3+} to Fe^{2+} state plays a major role in lipid peroxidation. Thus lipid peroxidation requires the presence of Fe^{2+} ion. As the concentration of iron increases, it finally accumulates in the liver. The tendency for the restoration of altered levels of iron and ferritin in rats treated with lycopene prior to the induction of hepatitis implicates the inhibitory effect of lycopene on lipid peroxidation chain reaction due to its well known antioxidant property [5].

Characteristic changes in the enzyme activities during hepatic injury induced by D-GalN/LPS have been reported earlier [31]. The increased activities of these enzymes indicate cellular leakage and loss of functional integrity of cell membrane in liver. A significant increase in the activities of AST, ALT, ALP, LDH and γ-GT in this study may be interpreted as a result of the liver cell destruction or changes in the membrane permeability indicating the severity of hepatocellular damage induced by D-GalN/LPS, which is in accordance with the previous reports our laboratory and others [21,52,55]. Pretreatment with lycopene in group 4 rats attenuated the increased activities of marker enzymes caused by D-GalN/LPS. This observation coincides with earlier findings [5] suggests that lycopene promotes parenchymal cell regeneration in liver, thus protecting membrane fragility thereby decreasing enzyme leakage and restoring the normal liver cell integrity. The histopathological analysis confirms the above findings.

5. Conclusions

Despite numerous studies on the health benefits of a carotenoid antioxidant lycopene, it has not been exclusively studied for its hepatoprotective potential particularly during hepatitis. The present study explores the ability of lycopene to protect the liver function during chemically induced liver injury in animal model. Liver damage induced by D-GalN generally reflects disturbances of liver cell metabolism resulting the derangement in the values of various metabolites of liver and in the activities liver marker enzymes. The results of the present investigation clearly reveal that the administration lycopene helps to restore the altered liver function during experimentally induced hepatitis. This protective ability of lycopene may be attributed by its well known antioxidant defense.

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