L-CARNITINE AMELIORATES IMMUNOLOGICAL-INDUCED HEPATITIS IN RATS

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يمكن استحداث إلتهاب الكبد المسبب مناعياً بواسطة منتج بكتيري هو السكر الشحمي المتعدد (LPS). إذ أن هذا الأخير يزداد أثناء الالتهابات الشديدة، أو النمو المفرط للبكتيريا أو انتقالها إلى مكان آخر. ويقوم السكر الشحمي المتعدد بتنشيط خلايا كوبفر مما يؤدي إلى المساهمة في إحداث الاصابات الكبدية عبر انتاج واطلاق المواد المسممة للخلايا، والسيتوكاينات الإلتهابية، وأنواع الأوكسجين المتفاعل. وفي هذه الدراسة ، تم استخدام مادة ل-كارنتين وهي مادة طبيعية مضادة للأكسدة وواقية مناعياً، وذلك للحماية من السكر الشحمي المتعدد المستحث بالالتهاب الكبدي. وتم تقدير محتويات الكبد من مادة جلوتاثيون ومادة مالونالدهيد ومادة أكسيد النيتريك ومادة 8-هيدروكسي ديوكسي جوانوسين. كما تم أيضاً تقدير النشاط المصلي لإنزيمات الكبد المختلفة. وعلاوة على ذلك، فقد تم تحديد التغيرات النسيجية المرضية (الهستوباثولوجية) للكبد. وأظهرت النتائج أن السكر الشحمي المتعدد (5 مغ/كغ مرة واحدة بريتونياً) يزيد معنوياً من مستويات 8-هيدروكسي ديوكسي جوانوسين، ومادة مالونالدهيد، ومادة أكسيد النيتريك، وتستنضب محتويات مادة جلوتاثيون في أكباد الجرذان المعالجة. كما أنها زادت من نشاط كل واسمات إنزيمات الكبد مما يدل على حدوث عطب خلوي كبدي هائل إذ ظهر أيضاً كعطب نخري في الأقسام النسيجية للكبد. وقد أدى إعطاء مادة ل-كارنتين (500مغ/كغ) قبل 3 ساعات من إعطاء السكر الشحمي المتعدد إلى الحماية من الخطورة القاتلة المستحثة من هذا السكر بشكل تام. كما أن ل-كارنتين منع الزيادة في محتويات الكبد من 8-هيدروكسي ديوكسي جوانوسين، ومادة مالونالدهيد ومادة أكسيد النيتريك . كما أنه أعاد مادة جلوتاثيون وحمى من العطب النخري لأنسجة الكبد حسبما تبين من عودة المستوى الطبيعي لإنزيمات الكبد والتحسن في هستولوجيا الكبد. وتوضح هذه البيانات بأنه يمكن استخدام مادة ل-كارنتين كعلاج مساند في المرضى الشديدي الإصابة وذلك لمعاكسة إلتهاب الكبد المستحث بالسكر الشحمي المتعدد.

Immunological mediated hepatitis can be initiated by bacterial product; Lipopolysaccharide (LPS). The later is increased during severe infection, bacterial overgrowth or translocation. LPS stimulates Kupffer cells. Activation of the kupffer cells contributes to the onset of liver injuries by producing and releasing cytotoxic agents, inflammatory cytokines and reactive oxygen species. In the present study, L-carnitine, a natural antioxidant and immunoprotective agent, is used to protect against LPS-induced hepatitis. Liver content of glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and the DNA adduct 8-hydroxydeoxyguanosine (8-HDG) are estimated. Serum activity of liver enzymes ALT, AST, and Gamma-GT, in addition to IL2 level are also estimated. Moreover, liver histopathological changes are determined. Results revealed that LPS (5mg/kg once i.p) significantly increased 8-HDG, MDA, NO and depleted GSH in the liver of the treated rats. It also, increased serum IL2 and activity of all the estimated liver enzyme markers indicating massive hepatic cellular damage as also shown as a necrotic damage in liver histological sections. LCR administered (500 mg/kg) 3h before LPS protected against LPS-induced lethality by 100%. LCR also prevented the increase in liver content of 8-HDG, MDA and

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NO. It rescued the depleted GSH and prevented the necrotic damage in the liver tissue as shown by normalization of ALT, AST and Gamma-GT as well as IL2 and a remarkable improvement in liver histology. These data suggest that LCR could be used as an adjuvant therapy in severely infected and septic patients to counteract LPS-induced liver hepatitis.

Key words: Immunological hepatitis, l-carnitine, LPS

Introduction

Lipopolysaccharide (LPS) is a major cell wall molecule of gram negative bacteria. It has been found to induce immunological mediated hepatitis (1). LPS has also been shown to induce stimulation of non-specific immune cells mainly macrophages, monocytes and antigen presenting cells (2). Kupffer cells, the resident macrophages in the liver, play a major role in the immunomodulation, phagocytosis and biochemical attack and defense (3). Activation of Kupffer cells contributes to the onset of liver injury by producing and releasing cytotoxic agents, inflammatory cytokines including tumor necrosis factor alpha (TNF), Interleukins (IL1, IL6), reactive oxygen species and inflammatory mediators such as nitric oxide (NO)(4,5). Some of these agents may attack the liver cells and lead to a significant injury to all the liver components. This, in tune, may lead to acute liver damage or dysfunction and if it is not controlled, it may stimulate the compensatory repair mechanisms leading to a chronic damage (6).

Different trials have been taken place to prevent Kupffer cell activation using several modalities such as kupffer cell eliminator, gadolinium chloride, or TNF antibodies (7). Other studies used the immunological liver injury model to develop promising hepatoprotective agents that can protect against the inflammatory squalls induced by kupffer cell activation such as melatonin (8).

Moreover, LPS has been reported to induce general oxidative stress condition as well as sepsis in animal models (9). Study the mechanism of LPS-induced toxicity, gains increasing attention from the scientific community. This may help to overcome its hepatic as well as lethal effects in severely infected, burned and post-operative patients even after administration of antibacterial agents. Moreover, Liver plays a major role in detoxification of LPS of intestinal derivation. Therefore, in cases of intestinal flora imbalanced growth or over growth, LPS-induced hepatic dysfunction may occur. Consequently, development of LPS counteracting agents was and still a hot issue in research. Fletcher *et al.,* (10) tried to develop an endotoxin neutralizing protein (ENP), a recombinant of anti-LPS factor, to detoxify LPS. In that regard, novel ideas came out to develop protective agents against LPS-induced hepatotoxicity such as stimulation of histamine (H2) receptors using H2-receptor knockout and histidine decarboxylase knockout mice (1). The authors reported that H2-receptor activation may protect against LPS hepatotoxicity.

Most of these trials showed a partial or cautious protection against LPS induced hepatotoxicity and searching for more promising , more safe and effective agents is still in demand.

L-carnitine (LCR) can be naturally synthesized in the body from lysine and methionine aminoacids. It is also found in diet (11). LCR has been found to play as a cofactor in the oxidation of long-chain fatty acids in the mitochondria. It also has an important role in carbohydrate metabolism as well as maintenance of cell viability (12). It has been reported that LCR can reduce the apoptotic levels of CD^{4+} and CD^{8+} (13). Moreover, it has shown as a powerful antioxidant and free radical scavenger (14).

In addition, many reports showed that LCR could protect against toxicity of several anticancer and toxic agents such as doxorubicin (15) and paraquat (16). It also showed a protection against magnetic field immunotoxicity (17) and testicular toxicity (18). Moreover, the observation that leukocytes are enriched in carnitines (19) first suggested that LCR and its congeners might regulate the immune networks.

These previous data stimulated our interest to investigate the possible protective effect of LCR against LPS-induced immunological hepatitis in rats.

Materials and methods

Chemicals:

Lipopolysaccaride (LPS), L-carnitine (LCR) were purchased from Sigma chemical Company street Louis, MO, USA. Thiobarbituric acid (TBA)

was a product of Fluka (Buchs, Switzerland). All the remaining chemicals were of the highest analytical grade commercially available.

2. Animals:

Male Swiss albino rats, weighing 200-250 g were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, KSA. Animals were maintained under standard conditions of temperature 24+1°C and 55+5% relative humidity with regular 12 h light: 12 h dark cycle and allowed free access to standard laboratory food (Purina Chow) and water.

3. Experimental protocol

1) Effect of LPS and /or LCR on rat survival.

a- The animals were divided randomly into six equal groups of rats (12 animals/group). Each group received a dose of LPS and the percentage mortality was assessed 24h later to determine the LD50 (20) of LPS. Doses of LPS are 0,1,3,5,7 and 9 mg/kg once i.p.

b- Thirty rats were divided into 4 groups. The first group served as a control and received Saline (6 rats); the 2nd group was given LCR (500 mg/kg i.p once, 6 rats). The third group was treated with LPS (5mg/kg i.p once, started with 12 rats and only 6 animals survived) and the fourth group received LCR then LPS after 3h (6 rats). Survival of all the animals was examined 24h later. Saline served as a vehicle to both LPS and LCR.

c- All parameters were assessed in the similar previous pattern in (b).

Blood sample were collected by direct withdrawal from the heart by means of heparinized syringes. Blood was left to clot and then centrifuged to separate sera.

The Animals were sacrificed by cervical dislocation and the livers were isolated, washed with saline, blotted dry on filter paper, and weight then 10% (w/v) homogenates of each sample was made in ice cold saline using homogenizer (VWR Scientific, Danburg , Com .USA).

1- Determination of GSH content in liver tissue

Tissue levels of acid soluble thiols, mainly reduced glutathione (GSH), were determined colourimetrically at 412 nm according to Ellman (21). Briefly, 0.5 ml of previously prepared homogenate was added to 0.5 ml of 5% trichloroacetic acid and after centrifugation at 3000 rpm for 5 min., the supernatant (200 µl) was added to a tube contains 1750 µl of 0.1 M Pot.phospate buffer, (pH 8) and 50 µl DTNB reagent. The tubes were mixed and the developed yellow color was measured against standard curve of reduced glutathione. Protein thilos (protein-SH) were expressed as µmol /g tissue.

2- Determination of lipid peroxides (MDA) in liver homogenates;

Tissue lipid peroxides level was determined as thiobarbituric acid-reactive substances (22). Tissue homogenates were prepared as previously mentioned above. Then, 0.1 ml of the homogenate was added to a tube containing 1.5 ml acetic acid (20%, pH 3.5) , 0.2 ml sodium dodecylsulphate,SDS, (8.1%), 1.5 ml TBA (0.8%) and 0.7 ml water against blank. The tubes were mixed and incubated in a water path at 95 ^{o}C for 60 min. using glass balls as condensers. Then, all the tube were cooled, centrifuged at 4000 rpm for 10 min. The absorbance was measured photometrically at 532 nm in the supernatant and the concentrations are expressed as nmol malondialdehyde (MDA) /g tissue

Using extinction coefficient of 156.

3- Determination of Nitric oxide (NO) in liver homogenates ;(23).

From the previously prepared liver homogenates, 0.5 ml was added to 0.5 ml of absolute ethanol then centrifuged at 4000 rpm for 10 min. Then to 300 µl of the supernatant 300 µl of vanadium chloride (VCl3, 0.8% in 1M HCl) was added. Then 300 µl of a mixture of Griess 1 and 2 reagents 1:1, and 100 µl of their solvents were added. Griess 1 reagent is composed of N-(1-naphthyl)-ethylenediamine (NEDD, 0.1% in distilled water) and Griess 2 is composed of sulfanilamide, 2% in 5% HCl. The mixture was left at room temperature 30-35 min then the color was measured spectrophotometrically at 540 against blank. Concentrations of NO were determined from a standard curve of different concentration of sodium nitrite. NO equivalent concentrations were calculated as nmol/g tissue)

4- Determination of 8-Hydroxy-2'-deoxyguanosine (8-HDG), a DNA adduct in liver-extracted DNA;

Liver DNA was extracted by phenol/ chloroform/isoamyl alcohol. Briefly 3ml of previously prepared liver homogenate was stilled down

by centrifugation at 1000 rpm for 5 min. then washed with phsophate buffered saline (PBS) pH 7.4. To the pellet, 2 ml of Tris-EDTA (TE) buffer [1M Tris-HCl pH 8 (100 ml) and 0.5M EDTA (100 ml) were mixed and completed to 300 ml with distilled water] was added. Then add 100 µl protinase K (10 mg/ml) and 240 µl 10% SDS (sodium dodecylsulphate), shake gently and incubate at 45°C in a water bath overnight. Then 2.4 ml equilibrated phenol was added, shaked and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a new tube and 1.2 ml of phenol then 1.2 ml of chloroform/isoamyl alcohol (24:1) were added, shaked for 5-10 min and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a new tube and 2.4 ml of chloroform/isoamyl alcohol (24:1) was added and shaked for 5-10 min. then centrifuged at 3000 rpm for 5-10 min. To the supernatant 25 µl of Sodium acetate (3M, pH 5.2) and 5 ml of cold absolute ethanol were added with gentile shaking, DNA will precipitate. The DNA was hooked out and washed with ethanol then dissolved in TE buffer and the concentration was obtained by determination of the absorbance at 260 nm. The purity of extracted DNA was determined by assessment of the ratio of the absorbance at 260/280. Purity of extracted DNA was above 97%. Extracted DNA was digested by DNase-1 (1U/1µg DNA). Digested DNA was subjected to determination of 8-HDG according to the protocol of the commercially available Kit by ELISA assay (24) (BIOXYTECH, 8-HDG-EIA Kit, ^{S}OXIS, Health Product. Inc. 6040 N Cutter Circle, Suite 317 Portland, OR 97217-3935 USA).

5- Assessment of IL2.

It was assayed in serum by ELISA according to the procedure described by the instructions of the commercial Kit (25) (Abcam Ltd, 332 Cambride Science Park, Milton Road, Cambridge CB4 OFW, UK).

6- Assessements of the activity of liver enzymes.

Alanine aminotransferase [ALT(26)], Aspartate aminotransferase [AST(27)] and Gamma-GT (28) were assessed according to the procedure described by the instructions of the commercial Kit (International Headquarters, Randox Laboratory Ltd, Diamond Road, Crumlin, Co, Antrim, UK).

7- Histopathological examinations;

Livers were collected and fixed in 10% formalin in phosphate buffer saline, (pH.7) for 24h at room temperature. Then, the tissues were embedded in paraffin wax and sections were cut at 5um thickness and stained with hematoxylin-eosin stains by routine procedures. A histopathologist who was unaware about treatments examined the coded slides by a light microscope and recorded the histopathological lesions and photographed them.

8- Statistical analysis

Data are expressed as (means± SEM). Statistical comparison between different groups were done by using one way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparisons test to judge the difference between different groups. Significance level was accepted at $P< 0.05$.

Results

A dose response curve is constructed to assess the LD50 of LPS. Injection of LPS in doses of 1,3,5,7 and 9 mg/kg once i.p (the animal survival was obtained 24h later) revealed that the dose of LPS that kills 50% of animals is 5 mg/kg Fig (1).

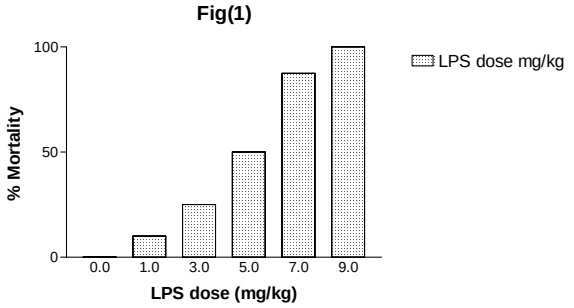


Fig 1. Effect of LPS on Survival rate in rats. Each dose of LPS was given i.p once and the number of rats alive/dead was counted 24h later. Number of animals /group=12

LCR was give in a dose of 500 mg/kg once i.p alone and 3h before LPS (5mg/kg). The results illustrated that LCR showed a 100% protection on the survival rate of rats when it was given in a dose of 500 mg/kg once i.p 3h before LPS (LD50) Fig (2).

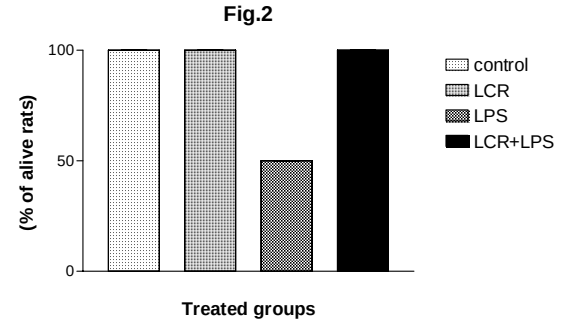
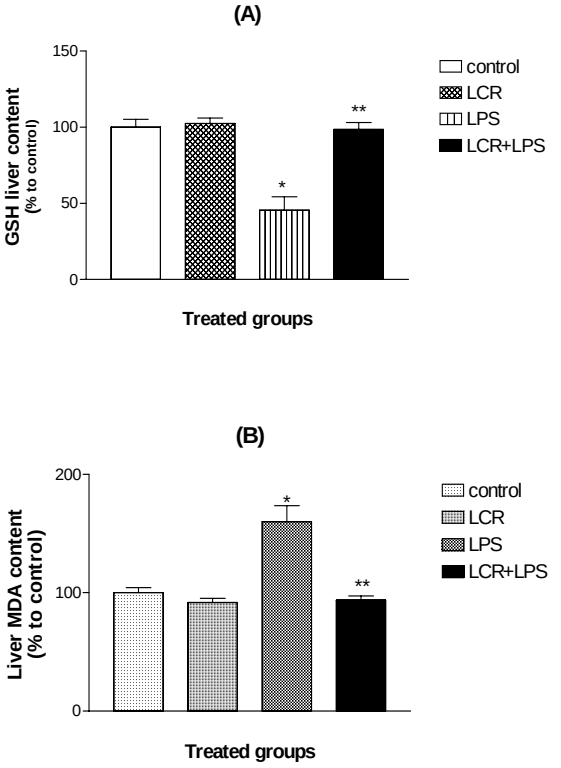


Fig 2. Protective effect of LCR on LPS-induced lethality in rats.
-LPS was given to rats once i.p in a dose of 5 mg/kg (LD50) and percentage mortality was assessed 24h later.
-LCR was given in a dose of 500 mg/kg once i.p 3h before LPS and percentage mortality was assessed 24h later.

The present results show that LPS (5 mg/kg once i.p) induces a significant depletion in liver glutathione (GSH) content by 54.5 % as compared to that of the control value (3.3± 0.17 µmol/g tissue) Fig (3A).



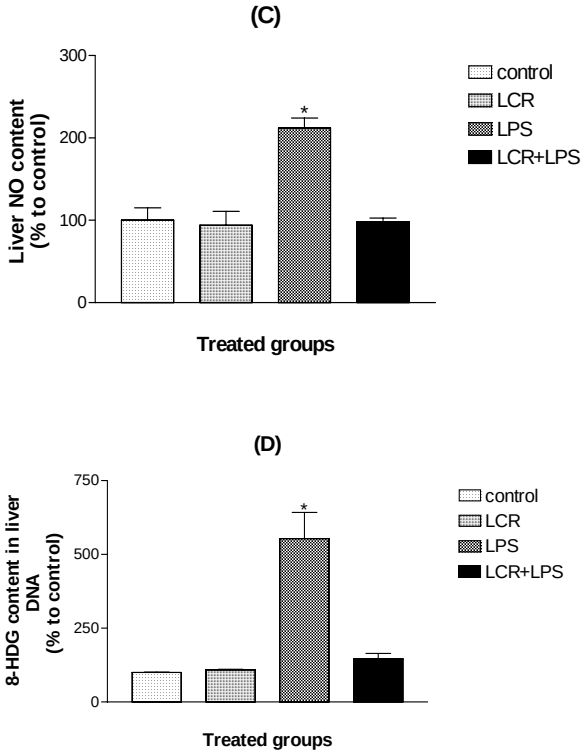


Fig 3. Effect of LCR and LPS on liver GSH, MDA, NO contents and 8HDG in rats.
GSH (A), MDA (B), NO (C) and 8HDG (D).
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-LCR was given in a dose of 500 mg/kg once i.p 3h before LPS.
-Control group was given saline. The number of animals/group = 6
-Statistical analysis was assessed using one way ANOVA followed by Tukey Kramer as post-ANOVA test.
* Significantly different from control at P<0.05
** Significantly different from LPS treated group at P<0.05

Administration of LCR (500 mg/kg i.p once) 3h before LPS restored completely the liver GSH content to get a similar value to that of the control animals Fig (3A).

LPS given to rats in a dose of 5 mg/kg once i.p (LD50) increases the liver content of malondi-aldehyde (MDA) by 60 % as compared to the value obtained from the control group (199.7±8.5 nmol/g tissue) Fig(3B).

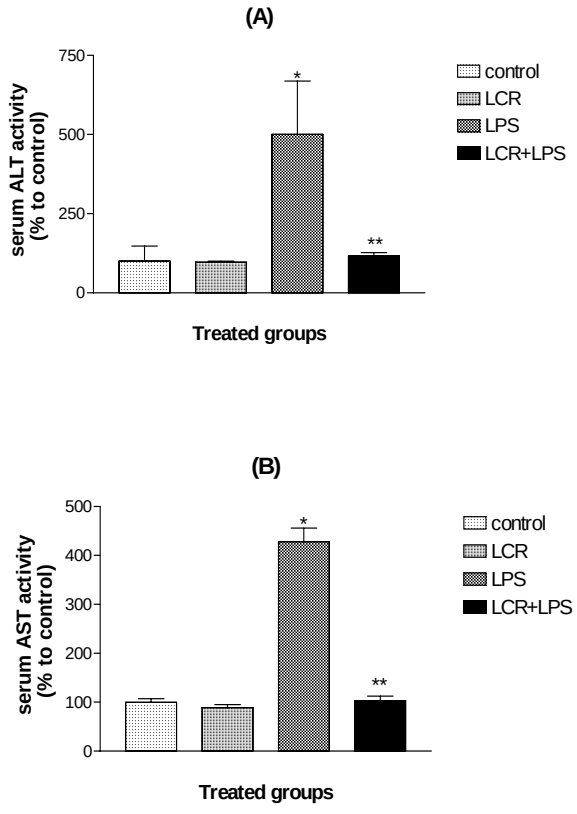
When LCR (500 mg/kg i.p once) administered 3h before LPS, it prevented the increase in liver MDA content and retuned it back to the control value Fig (3B).

Injection of LPS (LD50) once i.p to rats produced a marked induction of NO in the liver tissue, measured as nitrate/nitrite, to reach to 112 % increase as compared to the value of the control animals (0.66±0.1 µmol/g tissue, Fig (3C).

Upon injection of LCR (500 mg/kg i.p once) 3h before LPS, the liver NO content showed no significant change from that of the normal value Fig (3C).

The present results illustrate that LPS (5 mg/kg i.p once) induces liver oxidative DNA damage as shown by a significant increase in the 8-hydroxy-deoxguanosine (8-HDG), DNA adduct, in the liver-extracted DNA by 4 folds as compared to that of the control (0.56+0.01 ng/µg DNA) Fig (3D).

Pretreatment of LCR (500 mg/kg i.p once) 3h before LPS (LD50) resulted in a complete prevention of the increase in the level of 8-HDG in the liver-extracted DNA of the treated animals to get a similar value to that of the respective control Fig (3D).



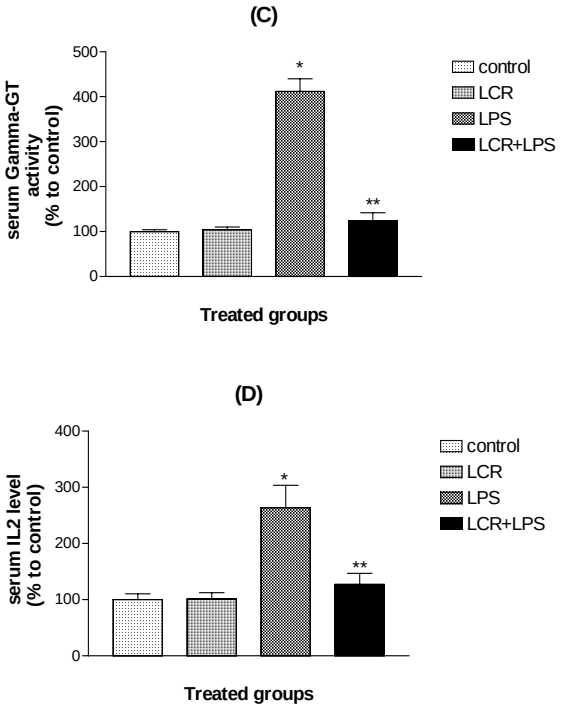


Fig 4. Effect of LCR and LPS on serum ALT, AST, GGT activities and IL2 level in rats.
ALT (A), AST (B), GGT (C) and IL2 (D).
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-LCR was given in a dose of 500 mg/kg once i.p 3h before LPS.
-Control group was given saline. The number of animals/group = 6
-Statistical analysis was assessed using one way ANOVA followed by Tukey Kramer as post-ANOVA test.
* Significantly different from control at P<0.05
** Significantly different from LPS treated group at P<0.05

Injection of LPS (5 mg/kg i.p once) induces a marked increase in the activity of the liver enzymes ALT, AST and Gamma-GT by 400%, 679% and 312% respectively as compared to the activity values respectively obtained from the control group (62±3, 68.9±5.1 and 4.9±0.2 U/L) Fig (4A,B and C)

Pretreatment of animals with LCR (500 mg/kg i.p once) 3h before LPS (LD50) normalized the activity of liver enzymes ALT, AST and gamma-GT in the treated rats Fig (4A, B and C).

The results illustrate that LPS (5 mg/kg i.p once) significantly increases the serum level of IL2 in rats

by 163% as compared to control group (11±1.1 pg/L) Fig (4D).

Pretreatment of LCR (500 mg/kg i.p once) 3h before LPS (LD50) completely abolishes the increase in IL2 level and obtained almost a normal value Fig (4D).

Histological examination; LPS induced a marked acute liver necrotic areas in the treated rats with severe congestion and infiltration of inflammatory cells (Fig. 5B).

When LCR was given 3h before LPS, it prevented the massive necrosis and significantly reduced inflammation in liver tissue showing residual mild dilated congested blood sinusoids (Fig.5C). Control normal histological structure of the liver is shown in Fig 5A.

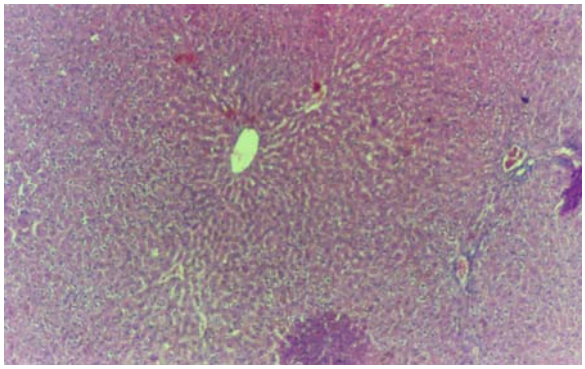


Fig 5A. Effect of LPS and LCR on the liver histology. (5A) a section from the liver (stained with routine H & E X=200) of a normal rat showing normal hepatic lobules and normal portal tract.

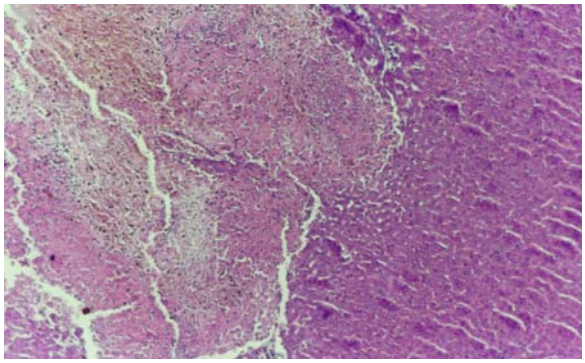


Fig 5B. A section from the liver (stained with routine H & E X=200) of LPS-treated rat showing a severe necrotic damage of hepatic cells with marked inflammation and immune cell infiltration.

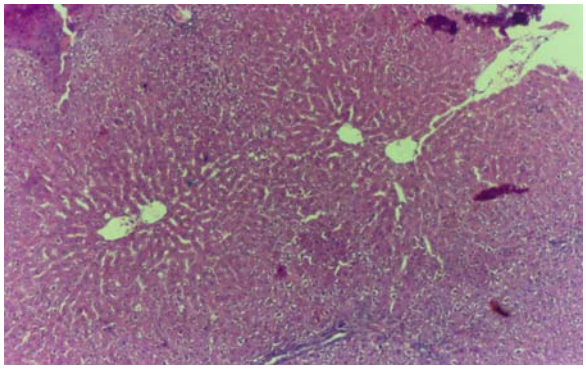


Fig 5C. A section from the liver (stained with routine H & E X=200) of LCR and LPS- treated rat showing almost normal hepatic lobules and normal portal tract with mild dilated congested blood sinusoids.

Discussion

Liver disease is increasing in incidence globally. Liver cirrhosis is the eight leading cause of death among disease in USA (29). In the face of this, the therapeutic repertoire for the treatment of liver diseases remains limited. Moreover, hepatology has not so far benefited from the synthesis of effective artificial organs unlike mechanical ventilation in respiratory medicine or dialysis in renal failure. In deed, orthotopic liver transplantation is the only effective treatment so far for severe liver failure although all limitations (30). Common to many etiologies of liver diseases is an inflammatory response. The key components of this response are chemokines such as IL1,2 and IL6. The later are small-protein inflammatory mediators (31). Hepatocytes play a central role in LPS derived from the intestine (32). Production of LPS by bacterial overgrowth, intestinal bacterial infection or translocation may stimulate immonological responses by hepatic cells (33). LPS has also found, in low concentration, to increase the toxicity of some hepatotoxic agents such as aflatoxin and alcohol (34).

LPS has also been shown to induce hepatic failure in murine models similar to what occurs in septic patients (35). Moreover, it is reported that murine livers have low number of major histocompatibility complex (MHC) receptors (36). This may get rats more vulnerable to LPS-immune consequences in the liver that may be benefited to search for more safe and effective hepatoprotective agents in case of immunological hepatitis.

In the present study, L-carnitine (LCR) is tested as a possible protective agent against LPS-induced hepatic injury.

Due to the diversity of LPS doses in the literature, a dose response of LPS is constructed. The LD50 of LPS is found 5 mg/kg i.p once and the animals were sacrificed 24h later. From a thorough search for safe LCR dose, a 500 mg/kg is selected to start with (18, 37). It was injected 3h before LPS (LD50) to test its possible protective effect on the lethality of LPS-treated rats and the animal survival was tested 24h later. Fortunately, the same dose of LCR revealed a 100% protection against LPS-induced lethality. Similar results were obtained before by Takeyama et al., (38) but the authors did not explain the mechanism of such protection. In the resent work the LPS-induced hepatitis is the target for protection. The present results show that LPS changed significantly the oxidative stress parameters. LPS depleted significantly liver glutathione (GSH) content and increased the liver malondialdehyde (MDA) content.

LCR restored the depleted GSH induced by LPS in rat livers. GSH exists in almost all cells in millimolar concentrations (39). It plays an important role in protecting cells against oxidative damage, maintaining a reducing environment in the cells and inactivating xenobiotics (40)**.** Inoue et al., (41) reported that carnitine could inhibit anticancer cellular damage by activation of mitochondrial superoxide dismutase. Moreover, Calabrese et al., (42) showed that acetylcarnitine increases the reduced GSH in patients with multiple sclerosis. Also**,** several reports illustrated that carnitines increased gastric content of reduced glutathione. Besides, they increase the enzymatic activity of gastric superoxide dismutase. Moreover, in the present study, LCR pretreatment, 3h before LPS, prevented the LPS-induced lipid peroxidation as shown by a marked decrease in MDA content in rat liver tissues. Similar results are obtained from many studies (18, 43, 44)**,** which showed that LCR restored GSH and prevented MDA formation. Furthermore, Atrosh et al., (45) have reported that the increase in GSH and protection against lipid peroxidation, are known to inhibit cellular damage.

Moreover, LPS induced nitric oxide (NO) in liver tissue. Pretreatment with LCR before LPS prevented completely the LPS-induced NO in the liver tissue. Inhibition of LPS –induced NO is considered a key mechanism in LCR protective effect against LPS-induced hepatitis. It is well known that NO is an inflammatory mediator particularly in immunological liver hepatitis (46).

Furthermore, LPS has been found, in the present work, to increase the oxidative DNA damage in liver cells as shown by the significant increase in DNA-adduct 8-hydroxydeoxyguanosine (8-HDG). The later is a highly selective DNA adduct for oxidative DNA which is among the most serious process indicating a massive cellular damage (47). In addition histopathological examinations of liver tissues of LPS-treated rats showed a massive acute necrotic damage with severe inflammation and immune cell infiltration. These results are supported by the significant increase in the inflammatory mediators NO content in liver tissue and IL2 in sera of LPS-treated rats as well as a significant increase in liver enzymes ALT, AST and Gamma GT. When LCR was given 3h before LPS, it prevented completely the increase in 8-HDG, and the increase in IL2 level as well as inhibition of NO induction. LCR also rescued the liver tissue from the acute necrotic damage and reduced significantly the inflammation and immune cell infiltration as compared to LPS-treated rats. These results are also supported by the capability of LCR to prevent the increase in liver enzymes ALT, AST and Gamma-GT. A recent report by Lee et al., (48) showed that the increase in Gamma-GT is a strong evidence and marker of liver oxidative damage. These effects may explain the mechanism of protection against the LPS-induced hepatitis. LCR showed similar protective effects against several insults and against several toxic agents such as carboplatin-induced myelosuppresion in rat bone marrow cells (49), doxorubicin and reactive oxygen intermediates (50).

Furthermore, endogenous LCR is synthesized in the liver, kidney and brain (51). Carnitine homeostasis is maintained by absorption from diet, a modest rate of synthesis and efficient renal reabsorption (52). Dietary LCR is absorbed by active and passive transfer across entrocyte membranes (52). The influence of LPS on liver synthesis and metabolism of LCR is not clear in the literature. Shankar et al., (53) reported that endothelial cells lose LCR upon administration of LPS leading to their dysfunction. This effect is prevented by exogenous LCR administration. Similar effect may occur in case of the liver content of LCR, a matter that needs to be evident by future investigation.

In conclusion, LCR has been found to protect

against LPS-induced hepatitis in rats through its antioxidant as well as anti-inflammatory effects. These data suggest that LCR could be used as an adjuvant therapy in case of septic shock and in severely infected patients as well as immunological-induced hepatitis.

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