ALPHA-LIPOIC ACID COUNTERACTS THE PROMOTED OXIDATIVE DNA DAMAGE IN THE LIVER OF SEPTIC RATS

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إن العدوى الطفيلية والفيروسية والمسرطنات الكيميائية هي من بين العوامل المسببة لسرطان الكبد. ومن المهم دراسة المواد التي تحدث ذلك وتنشره لتقييم مسببات وطرق منع مثل هذا المرض القاتل. يتم إبطال سمية السكر المتعدد الشحمي الناتج عن البكتيريا المعوية بواسطة الكبد. ولقد تبين أنه يحدث حالة من الضغط المؤكسد في الكبد. ولكن لم تتم دراسة قدرته على إحداث عطب مؤكسد للدنا (DNA) بشكل كامل، إذ قد تتسبب الزيادة في العطب المؤكسد للدنا وتشعب الخلية في بدء السرطان أو حتى انتشاره. وفي هذه الدراسة، تم اختبار قدرة السكر المتعدد الشحمي على حث مادة 8-هيدروكسي ديوكسي جوانوزين وهو أحد المواد المقربة من العطب المؤكسد للدنا في أكباد الجرذان. بالإضافة على ذلك تم تقييم التأثير الواقي المحتمل لحمض ألفا لايبويك أيضاً. والمؤشرات التي تمت دراستها هي محتويات الكبد من مادة جلوتاثيون، وفوق أوكسيدات الشحمية، وأكسيد النيتريك و 8-هيدروكسي ديوكسي جوانوزين في الدنا المستخلص من الكبد. كما تم تقييم نشاط كل من ALT و AST و GGT كعلامات دالة على وظيفة الكبد. كما تم تقييم مصل IL2 إضافة إلى فحص تشريحي للكبد. لقد تم إعطاء السكر المتعدد الشحمي بجرعات 1 و 3 و 5 و 7 و 9 مج/كجم مرة عن طريق الحقن تحت الجلد مع مراقبة حركة الفأرة بعد مرور أربع وعشرين ساعة تم إعطاء حمض ألفالايبويك بجرعات 50 و 100 و 200 مج/كجم مرة تحت الجلد وذلك قبل ثلاث ساعات من اعطاء السكر المتعدد الشحمي وقد وجد أن الجرعة التي تحدث موت النصف LD50 كانت هي 5 مج/كجم. كما أن السكر المتعدد الشحمي زاد من مستويات كل من 8-هيدروكسي دوكسي جوانوسين وفوق أوكسيدات الشحمية وأوكسيد النيتروز في الكبد. كما أنه تسبب في تنكرز والتهاب وترشيح في الحلية ، كما تبين ذلك من دراسة علم أمراض الكبد ومن الزيادة الواضحة في أنشطة كل من ALT و AST و GGT. وقد تسبب السكر المتعدد الشحمي في زيادة مستوى المصل لـ IL2 كما اتضح من إعطاء جرعة مقدارها 200 مجم/كجم من حامض الفاليبويك إلى حدوث حماية بدرجة 100٪ ضد مستحثات السكر المتعدد الشحمي القاتلة. كما أنه تسبب في منع مستحثات السكر الشحمي المتعدد لزيادة في 8- هيدروكسي ديروكسي جوانوسين في الدنا المتسخلصة من الكبد وكذلك محتويات الكبد من فوق أكسيدات الشحم وأكسيد النيتروز كما تسبب حمض الفاليبويك في انقاذ مستحثات السكر المتعدد الشحمي لفقدان مادة جلوتاثيون. وتسبب في تصحيح وظائف الكبد كما هو متضح من منع النشاط الزائد لـ ALT و AST و GGT مع تحسن واضح جداً في البنية النسيجية للكبد. وكذلك فإنه تسبب في منع زيادة مستوى المصل IL2. وكل هذه المعطيات تبين أن السكر المتعدد الشحمي يقوم بحث العطب التأكسدي لدنا الذي يمكن منعه بواسطة إعطاء حمض الفاليبويك مما يؤكد الدور الفاعل لحمض الفاليبويك كعلاج مساند لفرط اعتلال الكبد.

Viral, parasitic infections and chemical carcinogens are among the etiological factors of liver cancer. It seems important to study the initiating and promoting agents to evaluate the etiology and prevention of such life threatening disease. Intestine-derived bacterial product, lipopolysaccharide (LPS), is mainly detoxified by the liver. It has shown to induce a state of oxidative stress in the liver but its capability to induce oxidative DNA damage is not fully investigated. Increased oxidative DNA damage and rate of cell proliferation may initiate or even promote cancer. In the present work, the capability of LPS to induce 8-hydroxydeoxyguanosine (8-HDG), a specific DNA adduct for oxidative DNA damage, in rat livers is tested. Furthermore, a possible protective effect of alpha lipoic acid (ALA) is also assessed. Investigated parameters are liver contents of glutathione (GSH), lipid peroxides (MDA), nitric oxide (NO) and 8-HDG in

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the liver-extracted DNA. Serum activities of ALT, AST, and GGT as liver-function markers as well as serum IL2 are assessed. Moreover, liver histology is examined. LPS was given in doses of 1,3,5,7 and 9 mg/kg once i.p while, the rat mortality was examined 24hrs later. ALA was given in doses of 50,100 and 200 mg/kg once i.p 3h before LPS. LD50 of LPS is found to be 5 mg/kg. LPS increased the level of 8-HDG, MDA and NO in the liver. It also induced an acute liver necrosis and inflammatory cell infiltration as shown in liver-histopathology and in the significant increase in the activities of ALT, AST and GGT. LPS increased the serum level of IL2 as well. The dose 200 mg/kg of ALA revealed a 100% protection against LPS-induced lethality. It also, prevented the LPS-induced increase in 8-HDG in liver-extracted DNA, the liver contents of MDA and NO. ALA also rescued the LPS-induced GSH depletion. It corrected the liver function as shown by the prevention of the increases in the activity of ALT, AST and GGT with a remarkable improvement in liver histology. Moreover, it prevented the increase in serum level of IL2. These data illustrate that LPS can induce oxidative DNA damage which can be prevented by ALA suggesting a potential role for ALA as an adjuvant therapy in a plethora of liver disorders.

Key words: LPS, ALA , 8-HDG and oxidative DNA damage.

Introduction

Viral infection, alcohol and drug toxicity may elicit an interaction with the liver cells that may lead to hepatic damage (1). Bacterial endotoxin such as lipopolysaccharide (LPS) is among the agents that cause immunological stimulation of kupffer cells (2). Activation of the kupffer cells contributes to the onset of liver injuries by producing and releasing cytotoxic agents, inflammatory cytokines and reactive oxygen species (ROS). This may lead to severe oxidative damage of the liver cells (3). Oxidative stress in the liver cells may, in turn, lead to depletion of the natural reducing agents like glutathione (4). This process might give a hand to ROS to attack liver cells and cellular components like cell membrane, lipids, proteins and DNA (5). Toxic products like malondialdehyde (MDA), end product of lipid peroxidation, and protein adducts may be formed (6). Moreover, when ROS attack DNA to form a highly selective DNA adduct, 8-hydroxydeoxyguanosine (8-HDG), this will lead to a massive oxidative DNA damage (7). 8-HDG is removed from target DNA during its repair mechanisms. Continuous exposure to ROS may lead to depletion of DNA repair enzymes hence, increased chance for cellular responses that include apoptosis and/or necrosis (8). Severe necrotic damage stimulates compensatory regenerative processes and if disrepair occurs it will lead to cellular replacement of liver cells by deposition of collagen or cellular transformation (9), which may promote liver cirrhosis or even carcinogenesis (10).

Oxidative DNA damage and increased rate of cell proliferation are among the most common factors in developing cellular transformation leading eventually to precancerous or cancerous lesions (11).

Liver is the major organ for LPS detoxification that comes from bacterial intestinal flora (12). It increases in case of severe infection, burns, bacterial septicemia and septic shock (13). LPS is known to induce oxidative stress and liver damage (14) and to induce immunological hepatitis in animal models (15). Therefore, experimental study of oxidative DNA damage in target organs is a crucial aim to elucidate the relationship of liver cancer development and other harmful chemicals or drugs or even disease conditions. LPS has been found to increase the toxicity of alcohol and to promote carcinogenicity of aflatoxins (16). It seems that protection against chemicals induced oxidative DNA damage is important to limit carcinogenicity and liver toxicity. In that sense, LPS is used in the present work as a model to induce oxidative DNA damage and to study the possibility of protection using a strong antioxidant, alpha-lipoic acid (ALA).

Alpha-lipoic acid, also known as thioctic acid, is a naturally occurring compound that is synthesized by plants and animals, including humans (17). Alpha-lipoic acid contains two sulfur molecules that can be oxidized or reduced. This feature allows ALA to function as a cofactor for several important enzymes as well as a potent antioxidant (18). ALA has been reported to have hepatoprotective effect against many hepatotoxic agents such as lead (19), chloroquine (20) and sodium arsenate (21). It has also reported to prevent oxidative damage due to aging, diabetes or insulin resistance (22).

Moreover, ALA offers antiapoptotic effect on human bone marrow stromal cells (23) as well as anticancer effect (24). Doses of ALA as high as 1,200 mg/day (600 mg, 2 times/day) for 2 years or 1,800 mg/day (600 mg, 3 times/day) for 3 weeks did not result in adverse effects when given to patients

with diabetic neuropathy under medical supervision. There are no reports of toxicity from ALA overdose in humans. In individuals with diabetes and/or impaired glucose tolerance, ALA supplementation may lower blood glucose levels (25).

Therefore, the present study aims to use ALA as a protective agent against LPS-induced liver damage in septic rats.

Materials and methods

1. Chemicals:

Lipopolysaccharide (LPS) and Alpha-lipoic acid (ALA) were purchased from Sigma chemical Company (St. Louis, MO, USA). Thiobarbituric acid (TBA) is a product of Fluka (Buchs, Switzerland). All the remaining chemicals are 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 hrs light: 12 hrs dark cycle and allowed free access to standard laboratory food (Purina Chow) and water.

3. Experimental protocol

I) Effect of LPS and /or ALA on rat survival:

a- *Assessment of the LD_{50} of LPS*: The animals were divided randomly into six equal groups (12 rats/group). Each group of five received a dose of LPS (1, 3, 5, 7 and 9 mg/kg) and the control group received saline. The percentage mortality was assessed after 24hrs to determine the LD50 of LPS.

b- *Dose-response curve of the possible protective effect of ALA against LPS-induced mortality:* Sixty rats were divided into 9 groups.1) Control group included 5 subgroups (First subgroup received saline, the LPS-vehicle, while the 2nd subgroup given the vehicle of ALA (Ethanol: phosphate buffer saline, pH 7.2, 6:4) and the three remaining subgroups received ALA in doses of 50, 100 and 200mg/kg). 2) LPS - treated group 3) Three groups were given ALA (50, 100 and 200 mg/kg) 3hrs prior to LPS.

II- Protective effect of the selected dose of ALA (from the previous experiment) against the liver damage induced by LPS.

All the parameters were assessed in four groups later on as follows; 1) control group included 2 subgroups, one received saline and the other received ALA vehicle then saline after 3hrs. The total volume of i.p injection was about 0.25 ml.

Blood samples were collected from all groups after 24hrs of LPS injection 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. 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 (26). 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 potassium phosphate buffer (pH 8) and 50 µl of 5,5 dithiobis-2-nitrobenzoic acid (DTNB) reagent. The tubes were mixed and the developed yellow color was measured against standard curve of reduced glutathione. Protein thiols (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 (TBA)-reactive substances (27). 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 °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 nmole malondialdehyde (MDA) /g tissue using extinction coefficient of 156.

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

0.5 ml of liver homogenate was added to 0.5 ml of absolute ethanol then centrifuged at 4000 rpm for 10 min. To 300 µl of the supernatant, 300 µl of vanadium chloride (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 nm against blank. Concentrations of NO were determined from a standard curve of different concentration of sodium nitrite (28).

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

Liver DNA was extracted by phenol/ chloroform/isoamyl alcohol (29). Briefly 3ml of liver homogenate was stilled down by centrifugation at 1000 rpm for 5 min. then washed with phosphate buffered saline (PBS) pH 7.4. To the pellet, 2 ml of Tris-EDTA (TE) buffer (100 ml of 1M Tris-HCl pH 8 + 100 ml of 0.5M EDTA + 100 ml distilled water) was added. Then add 100 µl protinase K (10 mg/ml) and 240 µl of 10% SDS (sodium dodecylsulphate), shake gently and incubate at 45 ^{o}C in a water bath overnight. Then 2.4 ml equilibrated phenol was added, shaken 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 gentle 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 nm. 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 (BIOXYTECH, 8-HDG-EIA Kit, OXIS, Health Product. Inc. Portland, OR, USA).

5- Assessment of IL2:

It was assayed in serum by ELISA according to the procedure described by the instructions of the commercial Kit (Abcam Ltd, Cambridge, UK).

6- Assessments of the activity of liver enzymes:

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma-GT were assessed according to the procedure described by the instructions of the commercial Kit (Randox Laboratory Ltd, Antrim, UK).

7- Histopathological examinations:

Livers were collected and fixed in 10% formalin in phosphate buffer saline, (pH.7) for 24h at room temperature. The tissues were then 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 multiple comparisons test to judge the difference between different groups. Significance level was accepted at $P< 0.05$.

Results

I- Effect of ALA & LPS on rat survival:

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

A dose-response curve of the effect of different doses of ALA (50,100 and 200 mg/kg) on LPS (5 mg/kg) induced lethality was conducted. The results illustrated that a dose dependant protection of ALA

on the survival rate of rats treated with LPS reaching a 100 % survival when ALA was given in a dose of 200 mg/kg once i.p 3h before LPS as shown in Fig (2).

II- Protective effect of ALA against LPS-induced liver toxicity:

Regarding all the investigated parameters in this part of the study, ALA-vehicle showed no significant change from the control group that received saline.

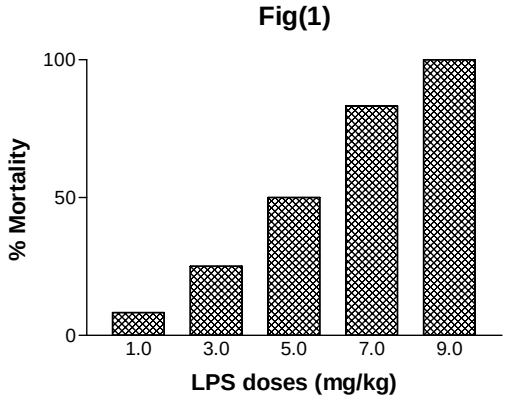

Fig 1. Effect of LPS on survival rate in rats Each dose of LPS was given i.p once and the number of rats alive/dead was counted 24h later. n =12

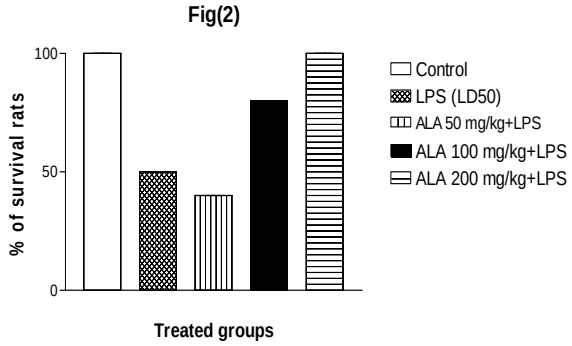

Fig 2. Protective effect of different doses of ALA on LPS-induced lethality in rats.
-LPS was given to rats once i.p in a dose of 5 mg/kg and percentage mortality was assessed 24h later.
-ALA was given in doses of 50,100 and 200 mg/kg once i.p 3h before LPS.
-Control group presents the mean of 5 subgroups (First was treated with LPS vehicle, 2nd group was given the vehicle of ALA and the remaining 3 groups received the three doses of ALA alone).

GSH:

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 as seen in Fig (3).

Administration of ALA (200 mg/kg i.p once) 3h before LPS restored completely the liver GSH content as shown in Fig (3).

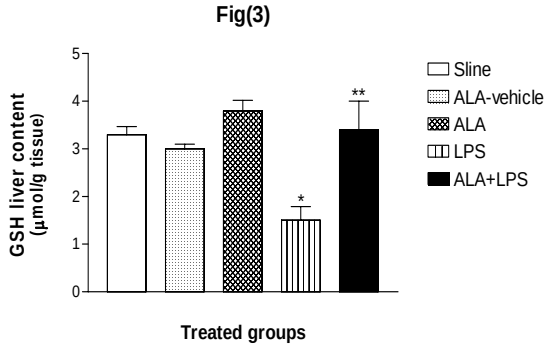

Fig 3. Effect of ALA and LPS on liver GSH content in rats.
-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at $P<0.05$
** Significantly different from LPS-treated group at $P<0.05$

MDA:

As indicated in Fig. 4, LPS given to rats in a dose of 5 mg/kg once i.p increased the liver content of malondialdehyde (MDA) by 60 % as compared to that of the control value.

When ALA (200 mg/kg i.p once) administered 3h before LPS, it prevented the increase in liver MDA content and returned it back to the control value as shown in Fig. (4).

Nitric Oxide (NO):

Injection of LPS once i.p to rats produced a marked induction of NO in the liver tissue, measured as nitrate/nitrite, to reach 112 % increase as compared to that of the control animals as indicated in Fig (5).

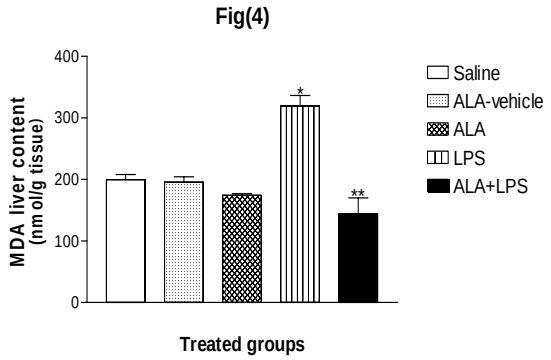

Fig 4.: Effect of ALA and LPS on liver MDA content in rats.
-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at P<0.05
** Significantly different from LPS-treated group at P<0.05

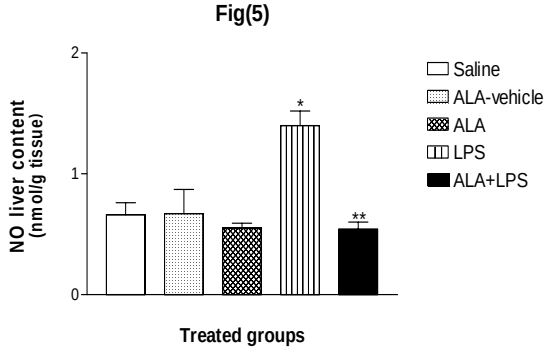

Fig 5. Effect of ALA and LPS on liver NO content in rats.
-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at P<0.05
** Significantly different from LPS-treated group at P<0.05

Moreover, as shown in Fig.(5), upon injection of ALA (200 mg/kg i.p once) 3h before LPS, the liver NO content showed a normal value.

8-HDG:

As illustrated in Fig.(6), LPS (5 mg/kg i.p once) induced a liver oxidative DNA damage as shown by a significant increase in the 8-HDG in the liver-extracted DNA by 4 folds as compared to that of the control.

Pretreatment with ALA (200 mg/kg i.p once) 3h before LPS resulted in a complete prevention of the increase in the level of 8-HDG in the liver-extracted DNA of the treated animals as indicated in Fig (6).

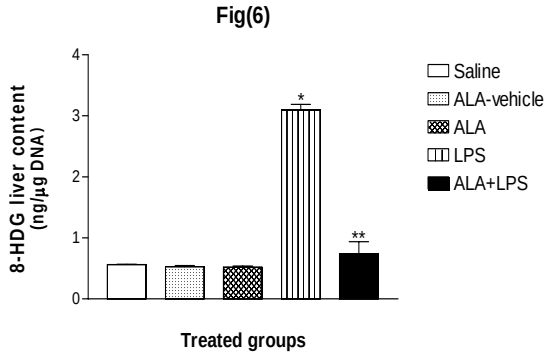

Fig 6. Effect of ALA and LPS on 8-HDG level in Liver-extracted-DNA in rats.
-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at P<0.05
** Significantly different from LPS-treated group at P<0.05

Assessments in rat sera:

Injection of LPS (5 mg/kg i.p once) induced a marked increase in the activity of the liver enzymes ALT, AST and Gamma-GT by 400%, 679% and 312%, respectively as compared to that of the control group as shown in Table (1).

Pretreatment of animals with ALA (200 mg/kg i.p once) 3h before LPS normalized the activity of liver enzymes ALT, AST and Gamma-GT in the treated rats as shown in Table (1).

Table 1: Effect of ALA and LPS on the activities of liver enzymes in rats.

Groups	ALT U/L	AST U/L	Gamma GT U/L
Saline	62±3	68.9±5.1	4.9±0.2
ALA-vehicle	60±2.1	65.8±6.9	5.1±0.4
ALA	61.5±2.7	59.3±5.2	4.8±0.4
LPS	310±21*	295±4.5*	20.2±.1*
ALA+LPS	69±6.1**	64.9±6.1**	6.8±.8**

-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at P<0.05
** Significantly different from LPS-treated group at P<0.05

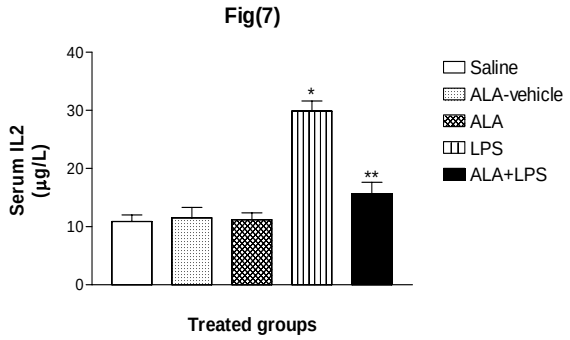

Fig 7. Effect of ALA and LPS on serum level of IL2 in rats.
-n = 6 except, LPS treated group n = 12.
-LPS was given to rats once i.p in a dose of 5 mg/kg and parameters were assessed 24h later.
-ALA was given in a dose of 200 mg/kg once i.p 3h before LPS.
-Control groups were given saline alone or the vehicle of ALA 3h before administration of saline.
* Significantly different from control groups at P<0.05
** Significantly different from LPS-treated group at P<0.05

IL2:

The results illustrate that LPS (5 mg/kg i.p once) significantly increased the serum level of IL2 in rats by 163% as compared to control group as indicated in Fig. (7).

Pretreatment with ALA (200 mg/kg i.p once) 3h before LPS completely abolished the increase in IL2 level and obtained almost a normal value as seen in Fig (7).

Histological examination:

A section from a control rat illustrates a normal liver histology as shown in Fig (8).

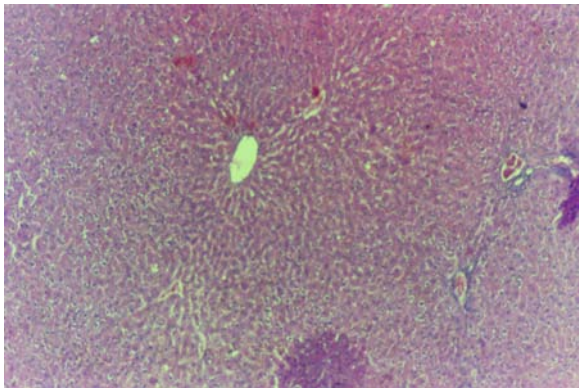

Fig 8. A liver section from a normal rat showing a normal hepatic histology (Stained with routine H & E, X=200)

LPS induced an acute necrotic damage to rat liver accompanied with dilation of blood sinusoids, acute inflammation and inflammatory cell infiltration as shown in Fig. (9 A).

As illustrated in Fig(9B), acute inflammation and numerous inflammatory cell infiltration is quite obvious in the liver section of LPS-treated rat.

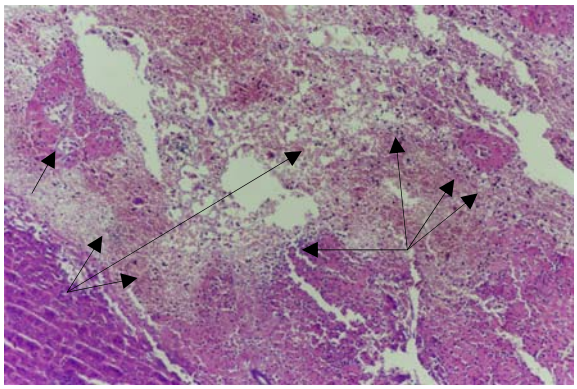

Fig 9A. A liver section from a LPS-treated rat showing severe acute necrotic damage (*triple arrow*), dilation of blood sinusoids (*single arrow*) and inflammatory cell infiltration (*quadrate arrow*). (Stained with routine H & E, X=200)

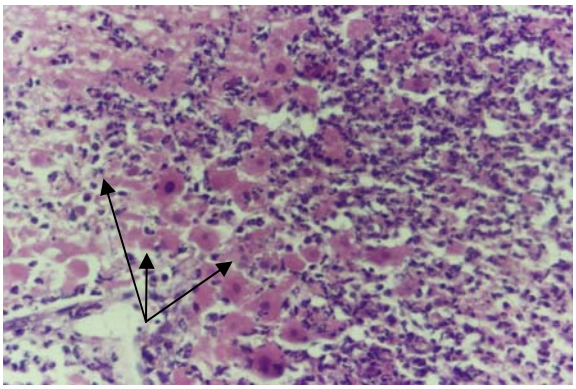

Fig 9B. A liver section from a LPS-treated rat showing acute inflammation and inflammatory cell infiltration (*triple arrow*) (Stained with routine H & E, X= 400).

Pretreatment with ALA (200 mg/kg i.p once) 3h before LPS (5mg/kg) significantly improved the histological structure of the liver. It prevented the features of acute necrosis and decreased markedly the inflammation and inflammatory cell infiltration as shown in Fig (10).

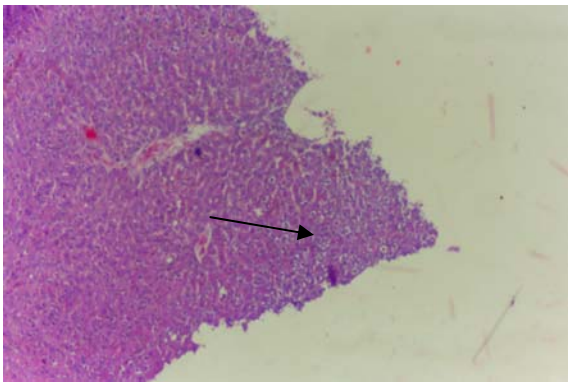

Fig 10. A liver section from ALA+LPS-treated rat showing no necrosis with mild inflammation and some inflammatory cell infiltration (*single arrow*) (Stained with routine H & E, X= 200).

Discussion

Inappropriate hepatic lipogenesis, hypertriglycridaemia, decreased fatty acid oxidation and muscle protein wasting are common in patients with sepsis or cancer (30). The adaptive immune system has evoked distinct responses against different pathogens or pathogen products such as LPS, but the mechanism(s) by which particular responses initiated is poorly understood (31). LPS induces human peripheral blood mononuclear cells (PBMC) to produce interferon-gamma (IFN-gamma). Monocytes play a mandatory accessory role in this process. LPS has been shown to amplify IL2-receptors expression on PBMC. Also IL1, a product of LPS-induced monocytes, potentiates the release of IL2-induced IFN-gamma (32). Furthermore, LPS has been documented to stimulate kupffer cells, the resident macrophages in the liver. Kupffer cell activation plays a central role in immunomodulation, phagocytosis and biochemical attack and defense (33).

These effects of LPS are accompanied by cytokine release of inflammatory mediators and most importantly accumulation of ROS (34). These responses lead to acute liver injury. Continuous exposure to such stresses may initiate oxidative DNA damage that in turn, promotes carcinogenesis (35). Protection against LPS-induced DNA damage is a crucial aim, therefore, the present study aims to use alpha lipoic acid (ALA) to protect against LPS-induced oxidative DNA damage in rat livers.

Due to the diversity of LPS doses in the literature, LD50 of LPS was determined. The LD50 of LPS was found to be 5 mg/kg i.p once. Also a dose-response curve of ALA against LPS-induced lethality was conducted. The dose of ALA (200 mg/kg i.p once 3h before LPS) that showed a 100% protection against the LD50 of LPS was selected to study the mechanism of that protection on the liver.

The present results showed that LPS induced a significant increase in liver lipid peroxidation, increased the content of NO and depleted liver GSH. These results point out an oxidative stress condition in the liver. The extent of this condition is documented in the production of a remarkable oxidative DNA damage in the livers of LPS-treated rats. This is shown as a significant increase in 8-HDG in the DNA extracted from the livers of LPS-treated rats.

DNA damage that is generated by oxygen-derived free radicals is related to mutagenesis, carcinogenesis and aging. Oxidative stress and oxidative DNA damage are involved in the pathogenesis of a wide spectrum of liver disease including cancer (36).

There are many mechanisms by which LPS can induce oxidative stress. LPS stimulates Kupffer cells, the resident macrophage in the liver.

Physiologically, macrophages serve as the first-line defense against invading pathogens by a) overproducing of superoxide anion ($NO2^-$) via activation of NADPH-oxidase localized in its plasma membrane, b) inducing the expression of inducible nitric oxide synthase (iNOS) and overproducing NO, and c) generating highly toxic peroxynitrite (ONOO-) to kill the invading pathogen (37).The excessive production of ROS is associated with inflammation and oxidative stress leading to the mediation of cyclooxygenase-2, prostaglandin-E2(PGE2) and matrix metaloproteinases (MMPs) (38). Moreover, Rushworth *et al.* (39) reported that LPS induces heme oxygenase-1 upon monocyte activation. Xu *et al.* (40) also reported the involvement of the up-regulated chemokine ligand 16/scavenger receptors that binds phosphatidylserine and oxidized lipoprotein in LPS-induced lethal liver injury via regulation of T-cell recruitment and adhesion. In addition, Wiegard *et al.,* (41) have reported that $CD4^+$,$CD25^+$ regulatory T-cells (Treg) can suppress $CD4^+$ T-cell stimulation by liver cells however, in response to LPS, liver cells can overcome the suppressive activity of Treg. Thus, liver cells may facilitate the transition from hepatic immune tolerance to hepatic inflammation. Furthermore, LPS has been shown to increase the levels of inflammatory mediators such as TNF alpha, IL2, IL1 and lots of ROS (42).

Strategies directed to counteract oxidative process could have a role in clinical medicine. There is evidence that oxidative stress acts as a major determinant of cell death. Many studies have reported favorable effects of antioxidants but few of them focused whether or not antioxidants could modulate oxidative DNA damage (43).

ALA, a strong antioxidant, has been reported to protect against many liver toxic agents (44, 45, and 46). In addition to the ability of ALA to protect against LPS-induced lethality, in the present study, it counteracted the oxidative condition induced in the livers of LPS-treated rats. Moreover, ALA prevented the LPS-induced GSH depletion and prevented the increase in MDA as well as 8-HDG in the livers when given in a dose of 200 mg/kg 3h before LPS.

GSH exists in almost all cells in millimolar concentrations **(**47**)** and plays an important role in protecting cells against oxidative damage, maintaining a reducing environment in the cells and inactivating xenobiotics **(**48**)**. Restoration of depleted GSH and prevention of the increase in MDA contents in the liver are key mechanisms in protection of ALA against oxidative stress induced by LPS. This could be useful in the explanation or understanding its ability to prevent the increase in LPS-induced oxidative DNA damage. Protective effect of ALA against LPS-induced 8-HDG, adds a wider spectrum of ALA activity as a powerful counteracting agent against cancer promoting condition in the liver (49).

Furthermore, ALA prevented the LPS-induced NO and prevented the increase of serum IL2. Inhibition of NO, as an inflammatory mediator as well as IL2 points out an anti-inflammatory effect of ALA against LPS-induced hepatitis. Similar effect was shown in a previous work (50). These results are supported by the ability of ALA to prevent the LPS-necrotic damage in rat livers and prevention of the increase in the activities of the liver enzyme markers ALT, AST and Gamma-GT. A recent report by Lee *et al.* (51) showed that the increase in Gamma-GT is a strong evidence and marker of liver oxidative damage.

In the light of the present and many previous results, it seems that the mechanism, by which ALA counteracted the LPS-induced liver damage, is mainly due to its antioxidant action. ALA has been shown to reduce markedly the hydroxyl radicals and to elevate the GSH rebounding system that protects GSH active enzymes from peroxidative damage (52). It is also reported that ALA saved the mitochondrial dysfunction and hence prevented the impaired ATP synthesis, the inefficient use of oxygen and the production of oxidants (53). Moreover, ALA has revealed a protective effect against DNA damage and apoptosis in insulin-secreting cell lines. Arivazhagan et al., (54) have also illustrated that ALA is functionally efficient in helping the cells to recover from oxidative damage (55).

In conclusion, these data suggest that ALA as a strong antioxidant can prevent the LPS-induced oxidative DNA damage as well as the squalls of oxidative and inflammatory stress in the liver. It also suggests the use of ALA as an adjuvant therapy against a plethora of hepatic inflammatory disorders.

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