

Research Article



The Role of Certain Factor in Treatment of Acute Hepatic Failure Induced by D-GalN/LPS Mediated by its Antioxidant Effects.

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ABSTRACT

Acute Hepatic Failure is the most serious liver disease worldwide and caused by D-GalN/LPS administration. The aim of this study is to assess the antioxidant effects of certain factor in rat liver treated with D-GalN/LPS. Thirty male Wistar rats were randomized into three groups of 10 animals. Normal control treated with saline, D-GalN/LPS group treated with (D-GalN (300 mg/kg) + LPS (10 µg/kg)), treatment group treated with a certain factor (100 mg/kg) + (D-GalN (300 mg/kg) + LPS (10 µg/kg)). We showed that D-GalN/LPS significantly increase the lipid peroxidation and ALT liver enzyme activity, whereas significantly decrease the antioxidant (GSH and CAT). We also observed a statistically significant decrease in MDA and ALT level with the certain factor treated animals, also it restores the depletion in GSH and CAT. In conclusion, this factor act as a powerful hepatoprotectant.

Keywords: D-GalN/LPS; Antioxidant; AHF; Oxidative stress.

INTRODUCTION

The liver is a vital organ of metabolism. Located in the upper right quadrant of the abdomen. The liver lobule is designed by parenchymal cells, named hepatocytes and nonparenchymal cells. In contrast to hepatocytes that occupy almost 80% of the total liver size and implement the majority of numerous liver functions, nonparenchymal liver cells, which donate only 6.5% to the liver size, but 40% to the entire quantity of liver cells, are contained in the sinusoidal compartment of the liver tissue. The walls of hepatic sinusoid are created by three diverse cell types: sinusoidal endothelial cells (SEC), Kupffer cells (KC), and hepatic stellate cells (HSC, previously known as fat-storing cells, Ito cells, lipocytes, perisinusoidal cells, or vitamin A-rich cells).¹ The liver has a wide range of functions, including metabolites detoxification, synthesis of protein and the manufacture of biochemicals essential for digestion.²

Acute liver failure (ALF) results in a horde of serious complications that frequently lead to multi-organ failure.³ D-Galactosamine (D-GalN)/lipopolysaccharide (LPS) is a widely used animal model of hepatotoxicity.⁴ Lipopolysaccharide (LPS) is a principle component of the outer membrane of Gram-negative bacteria and a prime target for recognition by the innate immune system.⁵ LPS increase expression and activation of NADPH oxidase, and the suppression of antioxidative enzymes involved in ROS clearance. NADPH oxidase activate production of reactive oxygen species (ROS).⁶ On the other hand D-galactosamine is a well-established hepatotoxicant that induces a diffuse liver injury closely similar to human viral hepatitis this followed by the regeneration process.⁷ As oxidative stress are a main pathogenic mechanisms

leading to liver damage,⁸ liver enzyme; Alanine amino transferase (ALT) are commonly used in the evaluation of patients with a hepatic disorder, because it found mostly in the liver and to some extent normally found in the blood, but when the liver is injured or diseased, it escaped ALT into the circulation.⁹

Malondialdehyde (MDA), was the most plentiful carbonyl products of lipid peroxidation. That produced by the reaction of the oxygen radicals with polyunsaturated fatty acid in phospholipids. MDA is reactive toward protein and DNA and form adducts.¹⁰

Catalase is a vital cellular antioxidant enzyme that protects against oxidative stress. It is initiate in the peroxisomes of most aerobic cells. It aids to defend the cell from toxic belongings of high concentrations of hydrogen peroxide H₂O₂ by catalyzing its conversion into molecular oxygen and water, without the creation of free radicals.¹¹

MATERIALS AND METHODS

Chemicals

Lipopolysaccharide, D-galactosamine was purchased from Sigma (St Louis, MO, USA). CAT, MDA and GSH kits were obtained from Biodiagnostic (Egypt). ALT kits were purchased from Human Biochemica Und Diagnostica mbH (Ggesellschaft Für).

Experimental animals

Male Wistar rats weighing 170±20 g were purchased from Beni-suef University Animal house. The rats, which were habitually fed and permitted to drink water freely, were exposed to experiments after 2 weeks of adaptation.



Animal grouping and treatment

Fourty Wistar rats were randomly divided into a normal group (saline) , D-GalN/LPS group (D-GalN (300 mg/kg) + LPS (10 µg/kg)^{12,13}, certain factor in a dose 100 mg/kg bodyweight.

D-GalN/LPS was administered intraperitoneally. Lipopolysaccharide, D-galactosamine administered once by intraperitoneal route. Lipopolysaccharide and D-galactosamine were prepared individually by dissolving the calculated required dose in calculating volume of saline as a vehicle.

Examination items

The blood sample was collected into sterilized tubes containing EDTA for plasma separation, and then centrifuged at 1500 rpm for 30 minutes. Plasma used for ALT activity assessment.

The rats were euthanized by carbon dioxide asphyxiation for tissue sampling, then liver was removed, and washed with normal saline. The liver tissue was washed with distilled water and homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 m M ethylenediaminetetraacetic acid (EDTA). The supernatant was separated by funds of cooling centrifugation at 5000 r.p.m for 20 min at 4 °C. The supernatant was used for the analysis of MDA, GSH and CAT.

Detection of biochemical indicator

Determination of ALT activity in plasma

Alanine aminotransferase was determined in blood plasma by kinetic method according to.¹⁴

Determination of Malonaldehyde (MDA)

Lipid peroxidation was evaluated in tissue liver homogenate by colorimetric method agreeing to.¹⁵

Determination of Catalase activity (CAT)

Catalase activity was examined in tissue liver homogenate calorimetrically according to the method of.¹⁶

Determination of Reduced Glutathione (GSH)

Glutathione (reduced) was tested calorimetrically in tissue liver homogenate according to.¹⁷

Statistical Analysis

Statistical analysis of the results was achieved using the (SPSS) statistical package for social sciences software (version 19.0).

Data were stated as Mean ± standard error mean (S.E.M).

All parameters were examined using one-way analysis of variances (ANOVA) and the Tukey test for post hoc multiple comparison.

The results were graphically represented by bar charts. A P value less than 0.05 was considered as statistically significant difference.

RESULTS AND DISCUSSION

Results

Liver function test

The obtained result as shown in Table (1) and Figure (1) revealed that, D-GalN/LPS significantly increased ALT activity in plasma of the animal if compared to healthy control ($P \leq 0.05$). On the other hand, certain factor significantly reduced the ALT activity in plasma of the animal if compared to D-GalN/LPS ($p \leq 0.05$).

Lipid peroxidation

The documented result in Table (1) and Figure (2) clarified that D-GalN/LPS administration produced oxidative stress that led to increase lipid peroxidation and MDA. Meanwhile the administrations of certain factor significantly decrease the lipid peroxidation product MDA in liver tissue.

Enzymatic (CAT) and nonenzymatic (GSH) antioxidant machinery

Table (1) demonstrates that, D-GalN/LPS administration produced highly significant depletion in the CAT enzyme activity and GSH liver tissue content related to normal control ($p \leq 0.05$). Treatment with certain factor significantly restores this depletion in CAT and GSH ($p \leq 0.05$). This could be attributed to their antioxidant and free radical scavenging property.

Table 1: Effect of certain factor on liver tissue levels of certain parameter in rats administered D-GalN/LPS induced hepatotoxicity and oxidative stress

Group / Parameter	Control	DGalN/LPS	Certain factor
ALT (U/L)	36.2±1.8	93±2.3 ^a	22.9±2 ^b
MDA(nmol/g)	80±7	240±36 ^a	40±4 ^b
GSH (mg/g)	195±9.8	70±3.3 ^a	186±3 ^b
CAT(u/g)	4800±120	2720±40 ^a	4255±140 ^b

Values represented as a mean± SEM of 10rats.

^aSignificant when compared to normal control group at $p < 0.05$.

^bSignificant when compared to D-GalN/LPS group at $p < 0.05$.

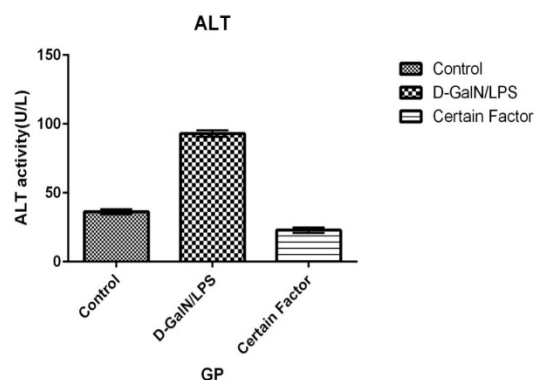


Figure 1: Effects of certain factor on the Activity of ALT compared to D-GalN/LPS treated animals.

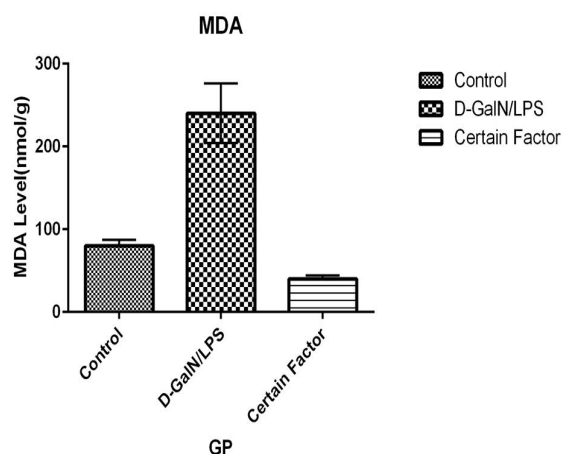


Figure 2: Effects of certain factor on the MDA content compared to D-GalN/LPS treated animal.

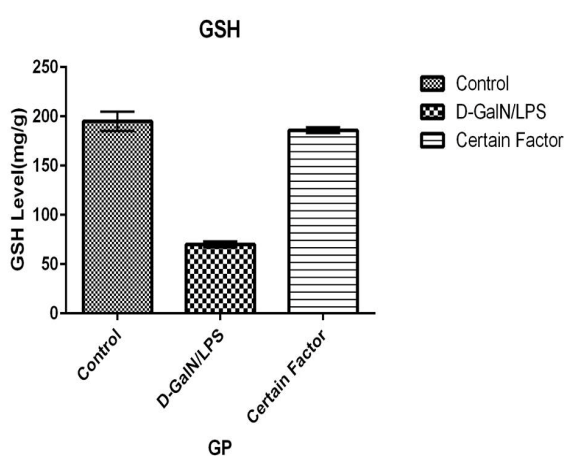


Figure 3: Effects of certain factor on the GSH content compared to D-GalN/LPS treated animal.

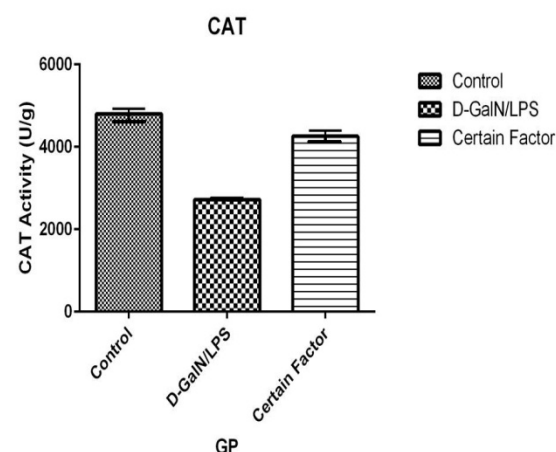


Figure 4: Effects of certain factor on the Activity of CAT compared to D-GalN/LPS treated animal.

DISCUSSION

Lipopolysaccharide (LPS) has an important role in the pathogenesis of D-galactosamine-sensitized rat's liver. The present study tested the liver function by assaying the alanine aminotransferase (ALT). Our result indicates

that plasma ALT activity was exaggerated upon D-GalN/LPS administration. These result agree with that published by¹⁸ who revealed that D-GalN/LPS administration induce ALT activity increase. This is due to oxidative stress produced by D-GalN/LPS that led to liver cell deterioration and leakage of intracellular enzyme ALT into blood circulation.

Certain factor is an antioxidant and anti-hepatotoxic property. Hence administration of Certain factor to D-GalN/LPS intoxicated rat in both prophylactic and curative group produce a highly significant decrease in ALT activity. The result in the present study are in consistent with the previous observation of¹⁹ who indicate that administration of Certain factor reduce ALT. Certain factor as antioxidant protect the liver cell from oxidative stress and preserve the liver cell membrane integrity so preserve the liver enzyme inside the cell so reduce the ALT in circulation.

D-GalN/LPS cause AHF due to the horde of oxidative stress produced. Whereas MDA is a product of polyunsaturated fatty lipid peroxidation, so these reactive species considered the oxidative stress markers. In the present study we show that administration of D-GalN/LPS produce a highly significant increase in MDA content in liver tissue. These result agree with²⁰ who indicate that MDA level increase to a high level in D-GalN/LPS induced liver injury.

Hence, certain factor has a powerful antioxidant property so it decrease the oxidative stress and decrease the free radical production and therefore decrease the lipid peroxidation and protect the liver cell. In this study, we indicate that, administration of Certain factor decrease the MDA tissue content. The obtained result are in harmony with²¹ who indicate that, Certain factor show antioxidant, free radical scavenging property so it act as hepatoprotective, , membrane stabilizing and anti-fibrotic agent. Accordingly certain factor may be useful as a therapeutic agent toward amelioration of AHF induced by D-GalN/LPS.

GSH and CAT considered as an indicator of antioxidant power in cell. While D-GalN/LPS produce a high amount of free radical that eradicated by enzymatic and nonenzymatic antioxidant power. In the present study administration of D-GalN/LPS produce a highly significant depletion in GSH and CAT. These data are parallel to that obtained by²² who found that, D-GalN/LPS deplete antioxidant machinery present in the liver of rats. This could be attributed to increase free radical production in liver of rats treated with D-GalN/LPS that lead to increased GSH and CAT consumption in attempt to neutralize the cellular function.

In the present study, we show that, Certain factor exert a highly significant restoration in enzymatic and non-enzymatic antioxidant system represented in GSH and CAT. This is due to the free radical scavenging property of certain factor, consequently oxidative stress significantly

decrease so increase the availability of the antioxidant machinery. The result in the present study are in consistent with the previous observation of²³ who demonstrate that, certain factor increase the availability of GSH and CAT, due to its antioxidant power.

CONCLUSION

In conclusion certain factor exhibit antioxidant, hepatoprotective, free radical scavenging, membrane stabilizing and protect the liver from deleterious effects of D-GalN/LPS, suggesting that it may be beneficial as a therapeutic agent toward improvement of AHF.

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