Research Article

Protective Effect of L-Carnitine, Atorvastatin, and Vitamin A on Acetaminophen Induced Hepatotoxicity in Rats

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ABSTRACT

This study aimed to assess the protective effect of L-carnitine, atorvastatin, and vitamin A on progression of acetaminophen induced hepatotoxicity in rats. Five groups of rats (8 rats in each group) were used. Group-1 received no treatment. Group-2 received a single dose of acetaminophen (1000mg/kg, intra-peritoneal (IP)) on fourth day. Groups (3, 4, and 5) were received oral doses of L-carnitine (500mg/kg), atorvastatin (10mg/kg), and vitamin A (5000 IU/kg) respectively for 7 days. On the 4th day a single dose of acetaminophen (1000mg/kg/IP) were administered to all these groups. Animals were sacrificed at day 8 and blood samples were collected by using cardiac puncture and used for analysis of ALT, AST, and ALP. The oxidative stress parameters glutathione (GSH) and malondialdehyde (MDA) were measured in liver. The rats in group-2 showed a significant elevation of serum ALT, AST, and ALP levels and a significant reduction of the GSH with a significant elevation of MDA levels in liver compared to group-1. All of these results were ameliorated by administration of L-carnitine, atorvastatin, and vitamin A. L-carnitine, atorvastatin, and vitamin A have protective effects against acetaminophen induced hepatotoxicity.

Keywords: hepatotoxicity, antioxidants, L-carnitine, atorvastatin, glutathione, malondialdehyde.

INTRODUCTION

Acetaminophen (Paracetamol) chemically named N-acetyl-p-aminophenol (APAP) is widely used both as an analgesic and as an Antipyretic agent[1]. Acetaminophen toxicity is one of the most common causes of poisoning worldwide[2]. Not surprisingly, acetaminophen remains a major cause of overdose and overdose-related liver failure and death in the United States and many other countries[3-4]. About 90% of ingested dose is metabolized through glucuronidation and sulfation pathway and 5% through CYP450 oxidase enzyme system[5-6]. Metabolism by CYP450 enzyme system produces a metabolite N-acetyl P-quinone immine (NAPQI) which is toxic to liver. In therapeutic dose, this is rendered ineffective by reduced GSH, an antioxidant compound in the liver and (NAPQI-reduced GSH) is excreted by kidney[7-8]. In acetaminophen overdose, sulfation and glucuronidation pathways become saturated. The amount and rate of formation of NAPQI is greatly increased, depleting body's reduced GSH stores and outstripping its capability to make new GSH. NAPQI then binds covalently with cells causing their death, resulting in liver dysfunction[6]. L-Carnitine is a known antioxidant agent, and has protective effects against lipid peroxidation. It is a cofactor in the transfer of long-chain fatty acid allowing the β-oxidation of fatty acid in the mitochondria[9]. L-carnitine is synthesized primarily in the liver but also in the kidneys and then transported to other tissues. It is most concentrated in tissues that use fatty acids as their primary fuel, such as skeletal and cardiac muscle. In this regard, L-carnitine plays an important role in energy production by conjugating fatty acids for transport into the mitochondria[10]. Atorvastatin is a member of statin drugs which are used primarily for lowering blood cholesterol and for prevention of events associated with cardiovascular disease. Like all statins, atorvastatin works by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), an enzyme found in liver tissue that plays a key role in production of cholesterol in the body[11]. Beyond their anti-hyperlipidaemic properties, statins exhibit many other biological activities, known as pleiotropic effects[12]. These includes anti-inflammatory, anti-proliferative, antioxidant and anti-apoptotic properties[13-15]. Vitamin A (also called retinol in mammals) is a fat-soluble vitamin. Human ingest two types of vitamin A: preformed vitamin A (retinol, retinal, retinoic acid) from animal source, and provitamin A (carotenoids, and β-carotene) from plants[16]. Vitamin A (Retinoids) have been considered as potent anti-inflammatory, anticoagulatory, antifibrotic, antioxidant and immunomodulating agents in animal models and in human diseases[17].

MATERIALS AND METHODS

Animals

Forty eight apparently healthy, albino wistar male rats with an average weight of 250 gm were selected for the study. The animals were housed in the animal house of The Higher Institute for Diagnosis of Infertility and Assisted Reproduction Techniques/AL-Nahrain University under standard laboratory conditions. The rats were

given standard rat pellets and water *ad libitum.* They were acclimatized to laboratory conditions for one week before starting the study. The study was carried out from March 2015 through June 2015. The protocol of the study has been approved by the Ethical Committee of the College of Medicine/AL-Nahrain University.

Study Design

Group-1 (8 rats) (Normal Control Group)

The animals were received no treatment, then sacrificed at day 8.

Group-2 (8 rats) (Acetaminophen Treated Group)

Each animal were given distilled water for 7 days orally. On the 4th day received a single intraperitoneal (IP) dose of acetaminophen 1000 mg/kg body weight[18-20], then sacrificed at day 8.

Group-3 (8 rats) (L-Carnitine Group)

Each animal were treated with 500mg/kg body weight/day of L- Carnitine[9,21-22] for 7 days orally. On the 4th day received a single IP dose of acetaminophen 1000 mg/kg body weight, then sacrificed at day 8.

Group-4 (8rats) (Atorvastatin Group)

Each animal were treated with 10 mg/kg body weight/day of atorvastatin[23-24] for 7 days orally. On the 4th day received a single IP dose of acetaminophen 1000 mg/kg body weight, then sacrificed at day 8.

Group-5 (8 rats) (Vitamin A Group)

Each animal were treated with 5000 IU/kg body weight/day of vitamin A[25-26] for 7 days orally. On the 4th day, received a single IP dose of acetaminophen 1000 mg/kg body weight, then sacrificed at day 8.

Preparation of Serum Samples

At the end of the study, the blood was drawn out from each animal under ether anesthesia by cardiac puncture using 5ml disposable plastic syringes. Blood samples were collected in sterilized plastic plain tubes (without anticoagulant), and left to stand for 30 min. at 25°C to get complete coagulation of blood samples, then centrifuged at 3000 rpm for 15 minutes[27] and the clear supernatant serum samples were aspirated by automatic pipette and transferred to eppendorf tubes and stored in a freezer set at -20°C for subsequent measurement of serum ALT and AST according to the method of Reitman and Frankel in 1957[28] using a ready-made kit for this purpose made in USA by Abbott, and ALP according to the method of Kind and King in 1971[29] by using a ready-made kit for this purpose made in USA by Abbott.

Preparation of Liver Tissue Homogenate

After blood collection, the animals were sacrificed. The abdominal cavity was immediately opened and the liver was dissected out of each animal, cut into two pieces and washed with sodium chloride 0.9% and excess moisture

was removed. One piece was quickly rinsed in chilled phosphate-buffered saline (PBS) at 4°C, blotted with filter paper and weighed. One gram piece homogenized with 10ml of PBS by using tissue homogenizer[30] for about 1min. at 4°C.

The resulting suspension was subjected to two freeze-thaw cycles for further break the cell membranes. After that, the homogenate was centrifuged for about 15 min. at a speed of 5000 rpm and temperature of (2-8)$^\circ$C.

The supernatant then collected carefully and stored at (-80°C) for the subsequent measurement of hepatic tissue MDA and GSH levels using ELISA technique.

Histopathological Examination

The liver of each animal in each group were prepared for histological examination according to the method of Bancroft and Stevens (1982)[31] with some modifications.

The specimens of liver tissue were fixed in 10% formaldehyde at room temperature, then passed through dehydration, cleaning, embedding with paraffin wax to form paraffin blocks which sectioned to 5 μm thickness using microtome and these sections where stained with Harris hematoxylin and Eosin (H&E) stains to be ready for histological examination.

Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Package for social Science) version (17), and Microsoft Excel Worksheet 2013. Crude data was analyzed to get mean and standard deviation. Student t-test was used to compare between two groups. P-value of ≤ 0.05 considered significant.

RESULTS

Effect of L-carnitine, atorvastatin, and vitamin A on serum ALT, AST, and ALP levels compared to acetaminophen induced hepatotoxicity in rats.

The serum ALT, AST, and ALP levels were significantly elevated in acetaminophen treated group compared to normal control group, indicating that acetaminophen induced hepatic damage. L-carnitine, atorvastatin, and vitamin A treatment showed a significant hepatic protection. Serum ALT, AST, and ALP results were showed in figures [(1), (2), (3)].

Effect of L-carnitine, atorvastatin, and vitamin A on oxidative stress parameters (GSH and MDA) compared to acetaminophen induced hepatotoxicity in rats.

Acetaminophen treatment significantly decreased the antioxidant GSH level (1.19±0.13) μg/ml and significantly increased lipid peroxidation end product MDA levels (7.1±1.3) nmol/ml compared to control group [GSH (2.68±0.1) μg/ml, MDA (3.81±0.21) nmol/ml in liver tissue homogenate. L-carnitine, atorvastatin, and vitamin A significantly elevated GSH levels [(2.34±0.21), (1.57±0.13), (2.1±0.13)] μg/ml respectively and significantly

attenuated the increase in MDA levels [(4.03±0.17), (5.49±0.22), (4.13±0.12)] nmol/ml respectively.

Histopathological Changes

Liver sections of rats in normal control group showed a normal histological structure of hepatic tissue that involved a thread like arrangement of hepatocyte cells figure (4). While the acetaminophen treated rats exhibited a wide area of necrosis with inflammatory cells infiltration, and hemorrhage compared to that observed in control group, figure (5). L-carnitine group showed a normal looking of hepatic tissue but with mild dilatation of sinusoid, slight shrinkage of hepatocyte, rare necrotic cells and mild inflammatory cells infiltration, figure (6). Atorvastatin group showed a normal looking of hepatic tissue but with minimal depletion of glycoprotein granules inside hepatocyte cells compared to acetaminophen group, figure (7). Vitamin A group showed a normal looking of hepatic tissue with mild inflammatory cells infiltration, figure (8).
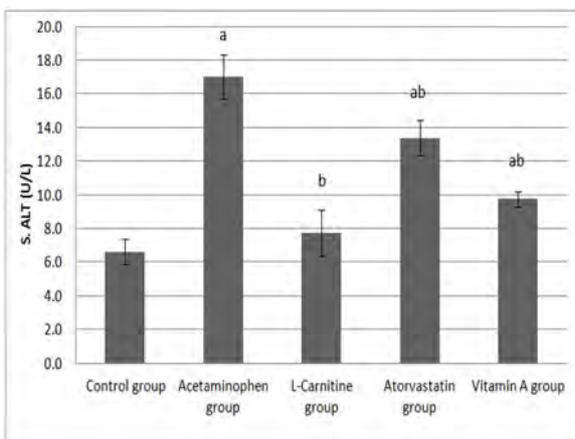

Figure 1: Effect of L-carnitine, atorvastatin, and vitamin A treatment on serum level of ALT compared to acetaminophen induced hepatotoxicity in male rats. a = significant difference with control group (P<0.05). b = significant difference with acetaminophen group (P<0.05).
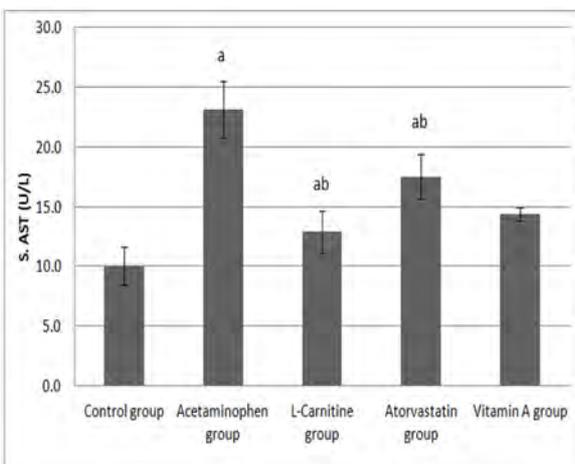

Figure 2: Effect of L-carnitine, atorvastatin, and vitamin A treatment on serum level of AST compared to acetaminophen induced hepatotoxicity in male rats. a =

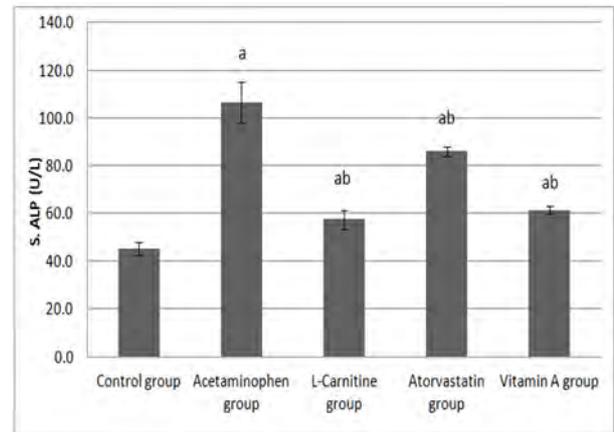significant difference with control group (P<0.05). b = significant difference with acetaminophen group (P<0.05).

Figure 3: Effect of L-carnitine, atorvastatin, and vitamin A treatment on serum level of ALP compared to acetaminophen induced hepatotoxicity in male rats. a = significant difference with control group (P<0.05). b = significant difference with acetaminophen group (P<0.05).
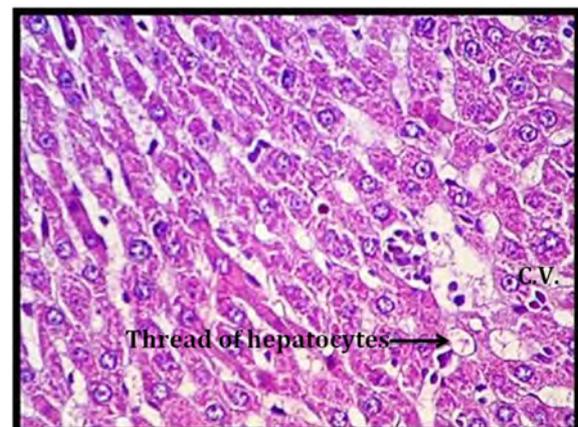

Figure 4: Normal control group shows normal hepatic tissue that involved a thread like arrangement of hepatocyte cells. H & E stain, (40X). C.V. = Central Vein.
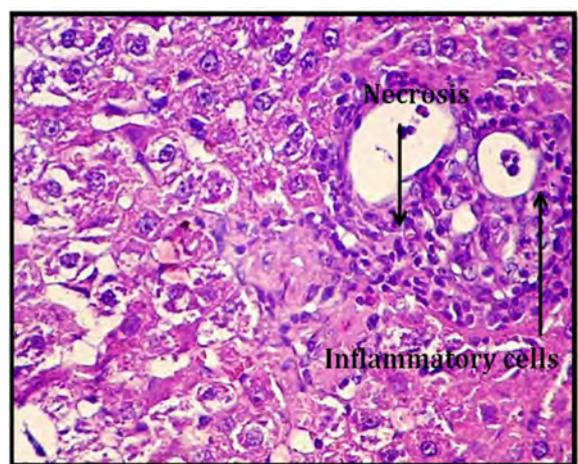

Figure 5: Acetaminophen group shows a wide area of necrosis with inflammatory cells infiltration. H & E stain, (40X).

Figure 6: (L-carnitine + acetaminophen) group shows a normal looking of hepatic tissue but with mild dilatation of sinusoid, slight shrinkage of hepatocyte, rare necrotic cells and mild inflammatory cells infiltration. H&E stain, (40X).
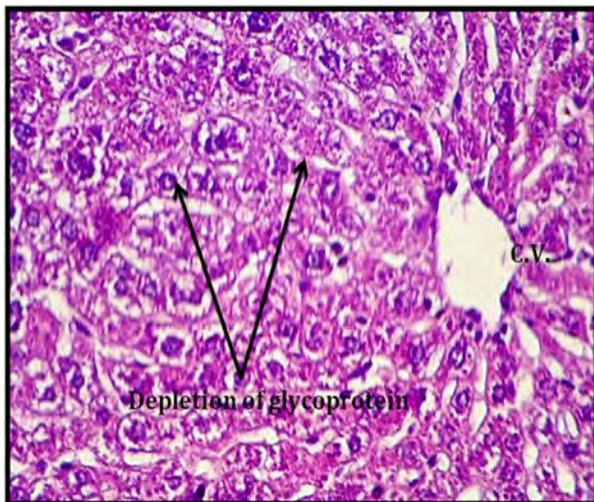

Figure 7: (atorvastatin + Acetaminophen) group shows a normal looking of hepatic tissue but with minimal depletion of glycoprotein granules inside hepatocyte cells. H&E stain, (40X). C.V. = Central Vein.
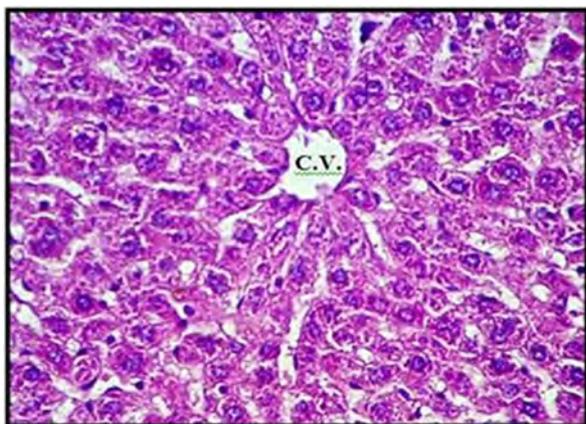

Figure 8: (vitamin A + acetaminophen) shows a normal looking of hepatic tissue with mild inflammatory cells infiltration. H&E stain, (40X). C.V. = Central Vein.

DISCUSSION

Oxidative stress appeared to be the main mechanism of acetaminophen induced liver injury[32-34]. It is an indicator of the damage that occurred due to an alteration of the balance between the oxidants and antioxidants in favor of oxidants. If this balance cannot be maintained in tissues, several pathological alterations extending to cellular damage would occur[21]. In this study, single dose of acetaminophen lead to hepatotoxicity that can be observed by the notable significant changes of biochemical parameters and supported by the histopathological findings in liver tissue. The elevated level of LPO end product MDA in liver of rats by acetaminophen suggested increased LPO causing liver injury and the antioxidant defense mechanisms were failed to mask excessive free radicals formations. These results are in agreement with many previous studies[35-40]. The results of present study demonstrated that the daily oral administration of each of L-carnitine, atorvastatin, and vitamin A for 7 days before and after the exposure to acetaminophen markedly ameliorated acetaminophen induced hepatic damage as shown in histopathologic examination and biochemical parameters. The ability of L-carnitine to significantly improve liver function enzymes may be due to its antioxidant effects and its capability to act as a radical scavenger, leading to protection of membrane permeability (prevention of the leakage of intracellular enzymes by its membrane stabilizing activity)[41]. Shebl and his coworkers illustrated that L-carnitine possess protective effect against sodium valproate hepatotoxicity by causing a significant reduction in serum ALT, AST, ALP, bilirubin, and lipid peroxidation levels that means it offer protection by maintaining the structural integrity of hepatocellular membrane against sodium valproate induced hepatotoxicity and oxidative stress[22]. The results of protective effects of L-carnitine in the current study are compatible with previous studies[42-44]. These protective effects of L-carnitine might result directly from antioxidant effects against oxygen radicals or from increased biosynthesis of enzymatic antioxidants, such as GSH and catalase[45]. The protective effect of L-carnitine on lipid peroxidation was done by decreasing hydrogen peroxide formation[46]. It can also improve antioxidant status and show free radical scavenging effect in rats[47]. Atorvastatin and other Statin drugs appeared to conferred 70-90% protection of liver against ischemia-reperfusion injury in obese animals with steatosis or non-alcoholic steatohepatitis[48]. Ali Zarei showed that oral atorvastatin is capable of improving liver function enzymes (ALT, AST, and ALP) and cause a significant decrement in their serum levels after induction of hypercholesterolemia in rats by administration of a fatty diet[24]. Previous studies illustrated that LPO and oxidative stress can severely elevated in patients with diabetes and hyperlipidemia[49-50] and atorvastatin can improve serum levels of liver enzymes by its antioxidant effect against liver damage (hepatocyte injury by free radicals). Free

radicals injure cell membrane as in hepatocytes. With destruction of hepatocytes membrane, the activity of hepatic enzymes increases which are normally in cytosol of cells and the elevation in the enzymes activity explains the type and degree of hepatic damage[51]. Yousef illustrated that atorvastatin treatment caused prevention of hepatic injury induced by carbon tetrachloride in a dose dependent manner with better hepatoprotective effects at a dose of 15mg/kg/day and these protective effects caused by atorvastatin antioxidant and free radical scavenging effects[52]. Moselhy and Sheikh illustrated that pretreatment of rats with atorvastatin 10mg/kg/day orally for 10days before the administration of a single IP dose of the anticancer drug doxorubicin (15mg/kg) preserved liver and kidney functions and histology deteriorated by the administration of doxorubicin via its antioxidant, anti-nitrosative, anti-inflammatory and anti-apoptotic mechanisms[53].

The results of the current study strongly support the notable antioxidant activity of vitamin A in previous reports[54-55]. Where vitamin A is one of the most known antioxidant defense systems together with vitamins C and E where they act as cofactors for numerous enzymes, preserve the cells against free radical injury by acting as free radical scavengers[56]. The protective results of vitamin A against hepatotoxicity in this study are compatible with other studies[57-59]. Vitamin A was supposed to had a structural function in preserving the integrity of the lipid membrane caused by its inherent structural properties and its physical and chemical properties (surface activity and participation in oxidation reduction reactions)[60]. Friday showed that the treatment of male rats with vitamins A (400 IU/kg/) or vitamin E (200 IU/kg) or both concomitantly with 4ml/kg of diesel fuel resulted in a significant decrease in the serum level activities of ALT, AST, ALP, GGT, cholesterol, triglycerides, LDL, VLDL and hepatic tissue MDA content, increase serum level of HDL, hepatic tissue CAT and SOD activities compared to that observed in rats received 4ml/kg of diesel fuel only and concluded that vitamins A and E have a comparable antioxidative and hepatoprotective effects against oxidative stress and hepatotoxicity induced by diesel fuel and that vitamin A is a less potent antioxidant than vitamin E in rats[59].

CONCLUSION

The results of this study suggested that oxidative stress play a major role in acetaminophen induced hepatotoxicity. L-carnitine, atorvastatin, and vitamin A have protective effects against acetaminophen induced hepatotoxicity and may have a therapeutic value in the treatment of acetaminophen induced liver damages.

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