Role of Protective Effect of L-Carnitine against Acute Acetaminophen Induced Hepatic Toxicity in Adult Albino Rats
(Light and electron microscopic study)
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ABSTRACT

Background: Acetaminophen, a widely used analgesic and antipyretic is known to cause hepatic injury in humans and experimental animals when administered in high doses. It was reported that toxic effects of acetaminophen are due to oxidative reactions that take place during its metabolism. L-carnitine is a cofactor in the transfer of long-chain fatty acid allowing to the beta-oxidation of fatty acid in the mitochondria. It is a known antioxidant with protective effects against lipid peroxidation. This study aimed to investigate the possible beneficial effect of L-carnitine as an antioxidant agent against acetaminophen induced hepatic toxicity in rats.

Material and Methods: Four rat groups (N=7 in each group). Group I is the control, group II received 500 mg/kg/ body weight of L-carnitine for 7 days by oral route, group III received 640/kg/ bw of acetaminophen by oral route, group IV acute acetaminophen group pretreated with L-carnitine for 7 days by gastric tube gavage tube. The liver of all rats were removed for investigation using light and electron microscopic studies.

Results: Acetaminophen caused massive centrilobular necrosis and massive degenerative changes. The electron-microscopic study showed few mitochondria, increased fat droplets and scanty smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER).These changes were reduced by L-carnitine pretreatment.

Conclusion: those results suggest that acetaminophen results damage in the liver as an acute effect and L-carnitine ameliorated the adverse effects of acetaminophen via its antioxidant role.

Keyword: Acetaminophen, L-carnitine, Liver & Rat.

INTRODUCTION

Liver diseases remain one of the serious health problems. Hepatic dysfunction due to ingestion of hepatotoxin is increasing worldwide

Acetaminophen (N-acetyl-P-aminophenol, (APAP), paracetamol) is one of the most commonly consumed drugs available on the market, but unfortunately it is also the most frequent cause of drug – induced liver failure world wide (Larson et al., 2005). About 500 fatal cases of acute liver failure are reported annually in the United States alone (Lee, 2004) and he added that acetaminophen toxicity is the leading cause of drug-induced acute liver failure in the U.S and Europe.

Acetaminophen, a commonly used analgesic and antipyretic drug causes fulminate hepatic necrosis in human and experimental animals when used in high doses. The biochemical mechanism by which
acetaminophen induced liver injury is believed to be due to its metabolic conversion to highly reactive intermediate N-acetyl p-benzoquinonimine (NAPQI) by cytochrome p-50 mediated oxidases, this metabolite binds covalently to essential hpatao-cellular protein and is known to be detoxified by glutathione (GSH) (Jaw and Jeffery, 1993). Overdose with APAP depletes cellular GSH, resulting dysfunction, ATP depletion, oxidative stress, DNA damage and oncotic necrosis of parenchymal cells (Kaplowitz, 2004; Jaeschke et al., 2011). The hepatocellular necrosis is accompanied by release of death-associated molecular pattern molecules (DAMPs), such as HMGB1, HSP70, and damaged DNA, which can activate non parenchymal cells, including Kupffer cells and neutrophils (Scaffidi et al., 2002; Bianchi, 2007; Martin-Murphy et al., 2010).

Other mechanisms include, the generation of the reactive oxygen species (ROS), sensitive transcription factor NF-kb, nitric oxide and lipid peroxides have been postulated to be the major factors involved in this liver injury. Reactive oxygen species (ROS) are considered to be involved in the liver damage induced by several conditions such as alcohol abuse, fibrosis/cirrhosis of various etiologies, hepatocellular carcinoma (HCC), ischemia/reperfusion (I/R) liver injury, paracetamol overdose, and viral hepatitis (Pablo, 2009). Therefore, prevention or impairment of oxidative stress constitutes a therapeutic target to be achieved for hepatoprotection. Different antioxidant strategies have shown to be useful to reduce oxidative stress and cell death in hepatocytes (Medina and Moreno-Otero, 2005). Dobrzyńska et al. (2010) found that L-carnitine protected liver cell membranes against oxidative modifications in ethanol-intoxicated rats through its ability to scavenge free radicals. Therefore, antioxidant activity of L-carnitine may play a role in the treatment of liver diseases.

L-carnitine (4-N-trimethyl ammonium-3–hydroxyl butyric acid) is a naturally occurring compound that is widely distributed in nature, especially in red meats and dairy products. L-carnitine is an endogenous mitochondrial membrane compound and it is a small water soluble molecule. It derived form two sources endogenous, synthesis low, levels of carnitine can be synthesized, primarily in liver and, to a lesser extent, en kidneys and brain, and from exogenous dietary sources (Al-Rejie et al., 2009; Mohamed et al., 2009). It is absorbed in the intestine and distributed to various tissues, with skeletal and cardiac muscle stores accounting for more than 98% of the total carnitine pool, L-carnitine presents in both plasma and tissue as free carnitine or bound to fatty acids as acyl carnitine derivatives (Lheureus and Hantson, 2009). Its main physical function is facilitating the transport of long chain fatty acids into mitochondria in order to enter the B- oxidation cycle (Sayed et al., 2010a&b). By combination with carnitine to form acyl caritine, acyl groups could be transferred form cytosolic coenzyme A on the outer surface of the mitochondrion membrane, than to the inner surface by exchange with free carnitine using an antiporte mechanism.
L-carnitine prevented in vitro human hepatocyte oxidative stress induced by hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). The protective effects of L-carnitine can possibly be mediated through its antioxidant potential. The elevated (peroxisome proliferator-activated receptor) PPAR-\(\alpha\) expression by L-carnitine plays an important part in the protective effect, which might contribute to the amelioration of lipid homeostasis, the improvement of antioxidant ability, and increased ATP in L-carnitine treated cells (Jin-Lian et al., 2012).

L-carnitine is an anti-oxidant that prevent the accumulation of end products of lipid peroxidation (Aydogdu et al., 2006). It is postulated that carnitine supplementation may increase the beta oxidation of some drugs (Lheureux and Hantson, 2009).

This study aimed to detect the possible protective effect of L-carnitine against the adverse effects of acetaminophen.

**MATERIAL AND METHODS**

(1) Animals:

Twenty eight adult male Sprague Dawley albino rats were used; their weights ranged from 120-180 gram. They were housed at room temperature and fed rodent chow and water. They were fasted 17 hours (4:00 pm - 9:00 am) before the experiment, but they allowed free access water.

(2) Experimental procedures and treatment:

Rats were kept in plastic cages during the experimental period. One week acclimatization period was allowed before initiation of the experiment. On the start of the 2\textsuperscript{nd} week experimental groups were divided into 4 groups (7 rats in each group).

**Group I**- The control group which was supplied with access water and ordinary rat chow.

**Group II** -(L-Carnitine group): the rats received 500 mg/kg L – Carnitine by oral route using a gastric gavage tube, for 7 days (Yapar et al., 2007).

**Group III** - Acute acetaminophen group: the rats received acetaminophen as a single oral dose 640 mg/kg dissolved in 50%propylene glycerol (5 mg/kg) using gastric gavage tube (Devi et al., 2005).

**Group IV**- Acetaminophen group pretreated with L. Carnitine for 7 days and supplied with ordinary rat chow. L-Carnitine 500mg/kg/day was administrated for 7 day by gastric gavage tube, followed by a single oral dose of acetaminophen (640 mg/kg b.w) and it was administrated by gastric gavage tube on the 8\textsuperscript{th} day. Tissue samples were collected 72 hours after acetaminophen administration.

(3) Tissue Sampling:

The animals were anesthetized by ether inhalation. A dorsal midline incision was done with dissection of the muscle to reach the liver

**Histological examination:**

For the light microscopic study: small pieces of liver from the different groups were fixed in 10% neutral buffered formaldehyde then processed to obtain 6 um – thick paraffin sections. Sections were stained with Harris haematoxylin and eosin stain and Masson's trichrome stain (Drury and Wallington, 1980) for routine morphological examination.
For the electron microscopic study the specimens were immediately fixed by immersion in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 0-4 °C and pH 7.3. The specimens were then washed for 1.5 hour with 3 charges of the same buffer for 2 hours (Merser and Birbeck, 1966). Ultra thin sections were then cut, stained and examined under a jeol 100 S transmission electron microscope.

RESULTS

Light microscopic results of the control group (GI) revealed normal appearance of hepatic architecture. The hepatocytes were arranged in anastomosing cords radiating from the central vein. The hepatic cords were separated from each other by bloods sinusoids lined by endothelial cells and Von kupffer cell. The hepatocytes were polygonal in shape with acidophilic cytoplasm and central rounded vesicular nuclei containing prominent nucleoli. Some hepatocytes were binucleated (Fig. 1).

Masson's trichrome stained section showed thin collagen fibers around the portal space (Fig. 2).

Electron microscopic examination of the control group (G.I.) showed hepatocytes with large rounded euchromatic nuclei with prominent nucleoli. The cytoplasm of the hepatocytes have many organelles distributed through it, where numerous mitochondria, cisternae of the rough endoplasmic reticulum and glycogen granules were observed. Kupffer cells were seen in the hepatic sinusoids with RBCs. These cells were also distinguished, located in the space of Disse and being characterized by present of vacuoles and lysosomes in their cytoplasm (Fig. 3 A&B)

L-Carnitine group (GII):

Liver sections of L-Carnitine treated group showed normal hepatocytes without noticeable structural changes if compared with the control group (Fig. 4). In Masson's trichrome stained section thin collagen fibers were detected around the portal space (Fig. 5).

Electron microscopic examination of this group appeared to be similar to the control group. The hepatocytes showed normal nucleus with normal peripheral chromatin, the cytoplasm contained many organelles such as: mitochondria, rough endoplasmic reticulum, smooth endoplasmic reticulum with plenty amounts of glycogen were realized.

The blood sinusoid contained red blood cells and kupffer cell, the cytoplasm of the kupffer cell contained, lysosomes, mitochondria and vacuoles (Fig. 6 A&B)

Acute acetaminophen group (GIII):

Light microscopic examination of the liver specimens of acute acetaminophen treated group (GIII), revealed variable structural changes of the hepatic tissue. Dilatation and congestion were observed in the central vein, bloods sinusoids and hepatic portal vein. Some hepatocytes were devoid of nuclei with vacuolated cytoplasm and pyknotic or karyolytic nuclei (Fig. 7A), while in some specimens the hepatocytes surrounding the central vein showed normal structure. Some spearing areas of hemorrhage and nodular necrosis were seen (Fig. 7A). Mononuclear cellular infiltration was observed around the portal spaces (Fig. 7B). Masson's trichrome
stained sections showed increased collagen fibers in the thickened portal tract with deposition around the wall of hepatic portal vein (Fig.8).

Electron microscopic examination of hepatocytes of acute acetaminophen treated group (GIII) showed obvious changes in the hepatocytes as increased, non membranous vacuolated areas replaced most of the cytoplasm. Few mitochondria were dilated with loss of their cristae. Increased fat droplets of different sizes were detected with scanty smooth (SER) and rough endoplasmic reticulums (RER). The nuclei were variable in size with irregular outline, others showed small nuclei (pyknotic) with peripheral localization of heterochromatin clumps. Abundent amount of collagen fibers were observed outside the hepatocytes (Fig.9A,B,C&D).

The Acute acetaminophen plus L-carnitine group (GIV)

Light microscopic examination of liver specimens of group IV, showed decreased dilatation and congestion in the central vein, blood sinusoids and the hepatic portal vein (Fig.10). However, some hepatocytes at the periphery of some lobules had deeply stained nuclei, while around the central vein the hepatocytes were apparently normal with vesicular nuclei (Fig.10). The mononuclear cellular infiltration could not be distinguished around the hepatic portal vein (Fig.10).

Masson's trichrome stained sections showed that the density of collagen fibers was more or less as control (Fig.11).

Electron microscopic examinations of hepatocytes of group GIV showed reduction in the structural changes as the majority of cells showed relatively normal ultrastructural. The mitochondria seemed to be normal, but some of them contained electron dense matrix with normal appearance RER. Plenty of glycogen flakes and granules with few lipid droplets could be also seen with collagen fibers (Fig.12A&B).

DISCUSSION

Liver is the main site of metabolism for most of drugs including acetaminophen, this organ is primarily exposed to toxic metabolites (Mitchell et al., 1973).

Although acetaminophen is considered as a safe analgesic, it has the potential of causing fatal hepatic necrosis when ingested in high dose (Jaw and Jeffery, 1993).

Acetaminophen is a commonly used and it is known to cause centrilobular hepatic necrosis upon overdose. Acetaminophen toxicity also accounts for many emergency hospital admissions and continues to be associated with high mortality (Aibo et al., 2010).

Previous studies showed that acetaminophen taken in overdose to the saturation of conjugation pathway led to formation of toxic relative metabolites. Free toxic metabolites are then covalently bound to macromolecules of cells leading to cellular necrosis. The toxic metabolite responsible for inducing liver damage is N-acetyl P-benzoquinone imine (NAPQI) which could bind to cellular proteins leading to centrilobular necrosis in the liver tissue. There are several factors could be involved in the mechanism and pathophysiology of acetaminophen hepatotoxicity.
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at the cellular level. Role of oxidative stress was reported to be one of the important factors in the development of the hepatic cell injury (Knight et al., 2001).

The present work showed acetaminophen induced hepatic injury as form of contrilobular necrosis and hemorrhage. Similar results were reported by many authors (Kinght et al., 2001; Sener et al., 2006; Martin-Murphy et al., 2010).

In the present work, the hepatocytes showing vacuolated cytoplasm with pyknotic nuclei and loss of cellular demarcation with increased cellular infiltration around the portal spaces post-acetaminophen treatment. Similar results were described by Kinght et al. (2001) and Sener et al. (2006) who reported that hepatocyte were swollen and eosinophilic particed pyknotic. The central and portal veins were dilated and congested with increased cellular infiltration specially around the portal spaces. Similar results were described by Mitchell et al. (1985) and Oak and Choi (1998) who reported that hepatocytes surrounding the central vein were swollen and sinusoids were congested with haemorrhage and area of necrosis around all the centrilobular regions.

Lores et al. (1995) reported that acetaminophen overdose caused the development of numerous cytoplasmic lesions in centrilobular hepatocytes. Detectable changes were observed in all the treated animals 6 hours after the drug administration. These changes include: swelling of the nuclear membranes, cytoplasm contained numerous vacuoles with variable degrees of glycogen depletion. These vacuoles were limited by single unit membrane. Furthermore, there were scattered clear vacuoles due to the lipoid droplets which were present in all the lobular zones, few hepatocytes bordering the necrotic areas had a shrunken appearance with condensed pyknotic nuclei or scattered fragmented particles. Added to condensation of the nuclear chromatin observed in the present work using the electron microscope some hepatocytes contained nuclei with irregular outlines and aggregated peripheral chromatin materials, the mitochondria are of variable sizes and shapes with ill-defined cristae, the rough endoplasmic reticulum was reduced and showed dilated cisternae. The smooth endoplasmic reticulum showed vesicular degeneration with multiple vacuoles in the cytoplasm. The blood sinusoids were dilated and congested by red blood cells and kupffer cells which obstructed their lumen and lies in contact with space of Disse, the kupffer cell displayed large irregular nucleus and vacuolated cytoplasm.

Results of the present study showed decreased dilatation and congestion in the central vein, blood sinusoids and the hepatic portal vein of live tissue of group pretreated with L-carnitine. The hepatocytes around the central vein were apparently normal with vesicular nuclei. The mononuclear cellular infiltration could not be distinguished around the hepatic portal vein, collagen fibers were more or less as control. The ultrastructure results showed reduction in the structural changes as the majority of the cells showed relatively normal appearance.
Oz et al. (2004) reported that hepatocytes noticed some obvious changes in the hepatocytes surrounding the central vein post-treatment with acetaminophen, since they were swollen and eosinophilic in appearance with cytoplasmic vacuoles, but antioxidant treatment led to less congested sinusoids with no sign of haemorrhage, the nuclei appeared pyknotic with very limited necrosis.

L-carnitine is a γ-3- methyl amino-β-hydroxyl fatty acid, which is an essential cofactor in mitochondrial respiration playing an important role in the transfer of long-chain fatty acids from cytosol to mitochondria. The acyl groups are then transferred from carnitine to coenzyme-A within the mitochondrion (Kelly, 1998). L-carnitine has a protective effect on lipid peroxidation by reducing the formation of hydrogen peroxide (Brass, 2000; Rani and Pannerselvam, 2002). L-carnitine could also improve antioxidant status in rats and showed free radical scavenging activity as well (Kalaiselvi and Pannerselvam, 1998; Rani and Panneerselvam, 2001).

L-carnitine have been found to offer protection against acetaminophen induced liver damage due to direct antioxidant effect by the mitochondrial energy production to facilitate the transfer of long-chain fatty acids from cytosol to the mitochondria, thereby playing an important role in the production of ATP. L-carnitine which increases ATP production in the myocardium in cisplatin-induced cardiomyopathy (Al-Majed et al., 2006).

Lipid peroxidation was suggested to be closely related to acetaminophen induced tissue damage (Senor et al., 2006).

CONCLUSION

In conclusion, findings of the present study showed once more that acetaminophen administered above the recommended therapeutic dose causes hepatic toxicity in rats, it was demonstrated that L-carnitine has a prominent protective effect against acetaminophen toxicity.

REFERENCES


Role of Protective Effect of L-Carnitine….


Figure (1): Light Photomicrograph of a liver section from the control rat (GI) showing cords of hepatocytes radiating from the central vein (c v) separated by blood sinusoids (S) which are lined by endothelial cells (E) Kupffer cells (KC), the hepatocytes have eosionophilic cytoplasm and rounded central nuclei (H&Ex200).

Figure (2): Light photomicrograph of liver section from the control rat (GI) showing little amount of collagen fibers in the portal space (PS) which contains portal vein (PV) hepatic artery (ha) and bile ducts (bd), with normal central vein (cv) (Masson's trichrome. X 200).
Figure (3) A: transmission electron photomicrograph from liver of the control rat (GI) showing, normal hepatocyte having rounded nucleus (N) with normal peripheral chromatin, mitochondria (m), rough (RER) and smooth (S.E.R) endoplasmic reticulum. Notice blood sinusoid contain red blood cells (RBCs). B: the hepatocytes (H) are separated by blood sinusoids (S) which contain kupffer cells (KC).
with large nucleus (N). Notice the cytoplasm of the kupffer cell has some lysosomes (head blue arrow), mitochondria (m) and vacuoles (thin blue arrow) (Ax 6000&Bx 8000).

**Figure (4)** Light photomicrograph of a rat liver section from L-carnitine treated group (GII), showing normal appearance of cell cords of hepatocytes radiating from the central vein (CV) separated by blood sinusoids (s) the hepatocytes have eosinophile cytoplasm and rounded central nucleus (N) (H& Ex200).

**Figure (5)** Light photomicrograph of a rat liver section from L.carintine treated group (GII) showing little amount of collagen in the portal space (ps) and the central vein (cv) (Masson's trichrome. X 200).
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Figure (6): Transmission electron photomicrograph from rat liver of L-carnitine treated group (GII) showing in (A): the hepatocyte (H) with normal distribution euchromatin nucleus (N) having prominent nucleolus (n). The cytoplasm is rich in normal mitochondria (m) and glycogen granules (G). In (B): notice that blood sinusoid contains (RBCs) and kupffer cells (kc) with large nuleus (N) (A&Bx 8000).

Figure (7) Light photomicrograph of a rat liver section from acute acetaminophen treated group (GIII) showing in (A) expanded portal space (ps), the surrounding hepatocytes showed degeneration with loss of demarcation (black arrow), multiple vacuolated cytoplasm (V) pyknotic nuclei (P) can be seen in some hepatocytes, the central vein is congested and dilated (CV), large area of hemorrhage (*) and
nodular necrosis (▲). The Blood sinusoids (S) are dilated and congested. In (B): inflammatory cellular infiltration (arrow) are seen around the portal tract (PT) (H&.Ex200).

**Figure (8)** Light photomicrograph of a rat liver section from acute acetaminophen treated group(GIII) showing expanded portal space (ps), dilated of portal vein (pv), new bile duct proliferation (Bd), increased deposition of collagen fibers (C) around the portal tract. The surrounding hepatocytes (H) show degeneration and loss of demarcation (d) with vacuolated cytoplasm (v) (Masson's trichrome x 200).
**Figure (9):** Transmission electron photomicrograph from a rat liver of acute acetaminophen treated group (GIII) showing, (A): a hepatocyte with aggregated peripheral chromatin in the nucleus and large non membranous vacuolated areas (V) in the cytoplasm, fat droplets, scanty of RER, SER and mitochondria are seen. (B) showing, numerous fat droplets (F), swollen mitochondria (m) with ill-defined cisternae, notice irregular outline of the nuclear membrane with heterochromatin in the nuclei (N) and depleted glycogen granules (G). (C) showing marked shrunken (pyknotic) nucleus (N) with few organelles (D) showing presence of collagen bundles (c) of different sizes that encircled the blood sinusoid (s). (A,C,DX6000&BX10000)
**Figure (10):** Light photomicrograph of rat liver section in acute acetaminophen and L. carnitine group (GIV) showing normal cords of hepatocytes radiating from slightly dilated centre vein (CV), separated by blood sinusoids (S), notice minimal cytoplasmic vacuoles (V) at the periphery of the lobules, so they are more or less similar to control (H&Ex200).

**Figure (11):** Light photomicrograph of a rat liver section in acute acetaminophen and L. carnitine treated group (GIV) showing slightly dilated and congested portal vein (pv) with little amount of collagen fibers surrounding the portal vein, the hepatocytes (H) showed vacuolated cytoplasm (v) (Masson's trichrome x 200).
Figure (12): Transmission electron photomicrograph from rat liver of acute acetaminophen and L-carnitine treated, group (GIV), (A) showing relatively normal ultrastructure of the hepatocytes with more or less normal rounded nuclei (N) and nucleoli (NU). (B) notice that the mitochondria (m) appear slightly swollen and show variability in size and shape. The rough and smooth endoplasmic reticulum (RER)/(SER) are mild reduced.

Notice, normal amount of glycogen and the presence of small vacuoles (v). (A x6000&B X 25000)
Role of Protective Effect of L-Carnitine in the White Rats

The protective effect of L-carnitine against the acute effect of aspirin-induced liver damage in adult white rats (a histological and electron microscopic study)

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Abstract:

Liver damage is one of the most severe dangers to which humans are exposed. Aspirin is one of the most common drugs used as analgesics and anti-inflammatory agents. Use of this drug at high doses, such as occurs in some cases, or at the recommended doses in cases of prolonged fasting, may lead to severe liver and kidney damage.

Therefore, this study was conducted to investigate the beneficial effect of L-carnitine as an antioxidant against the liver damage caused by aspirin in adult white rats.

A total of 82 male rats weighing between 0.81 and 0.21 kg were randomly divided into four groups. Group 1 was a control group, Group 2 was a group that received aspirin (0.41 mg/kg of body weight) by mouth once, Group 3 was a group that received L-carnitine (0.011 mg/kg of body weight) by mouth for 7 days, and then received aspirin (0.41 mg/kg of body weight) by mouth once. Group 4 was a group that received L-carnitine (0.011 mg/kg of body weight) by mouth for 7 days, then received aspirin (0.41 mg/kg of body weight) by mouth once in the eighth day.

At the end of the study, the rats were anesthetized and liver samples were collected and prepared for light and electron microscopic examination.

Light microscopy showed an increase in the liver cell blood vessels, liquefaction of the liver cell nuclei in the form of spaces, inflammation cells surrounding the blood vessels, and widening of the biliary ducts with collagen fibers around the blood vessels and an increase in their thickness.

Electron microscopy revealed the accumulation of fat in the liver cells, changes in the size and shape of the mitochondria, and a decrease in the endoplasmic reticulum network, and indentation of the cell nuclei in the liver cells. There was also the presence of bundles of collagen fibers inside the biliary ducts.

The results showed the toxic effect of aspirin on the liver and the role of L-carnitine in preventing the complications that occur during the treatment with this drug. We concluded that the use of L-carnitine (aspirin) can protect the liver from the toxic effects during treatment with aspirin.