Role of β-Hydroxy-γ-Trimethylammonium Butyrate (L-carnitine) and Ubiquinone (CoQ10) in Combating the Deteriorative Effect of Halogenated Alkanes in Liver

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ABSTRACT

Liver cirrhosis is one of the most dangerous health problems which results from various disorders that damage liver cells over time. The purpose of the current report was carried out to provide information about the liver injury induced by halogenated alkanes (CCl4), and to determine the influence of administration of L-carnitine or/and CoQ10 as prophylactic agents against that injury. The study was carried out on 80 adult male albino rats divided into eight groups, 10 animals of each, as follows: four normal groups (control, treated with L-carnitine, CoQ10, and a combination of L-carnitine and CoQ10) and four cirrhotic groups treated with CCl4 (control, pretreated with L-carnitine, CoQ10, and a combination of L-carnitine and CoQ10). Liver cirrhosis was induced by s.c. injection of a single dose of CCl4 (1 ml/kg). L-carnitine (50 mg/kg/day) was given i.p. for four successive days 24 hours before CCl4 injection, and CoQ10 was given as single i.p. dose (200 mg/kg) 24 hours before CCl4 injection. Animals were sacrificed 24 hours after CCl4 injection, blood samples were withdrawn and liver tissue samples were homogenized. The studied parameters were: hepatic reduced glutathione (GSH), lipid peroxides, ALT and AST activities, total protein, lactate dehydrogenase (LDH) activity in both serum (with its isozymes) and liver tissues. The injection of CCl4 produced a significant decrease in reduced glutathione content and total protein levels in serum and liver cells. However, there was a significant increase in serum ALT and AST activities, lipid peroxides, serum and hepatic total LDH and serum LDH isoenzymes. On the other hand, groups treated with L-carnitine or/and CoQ10 (prior to CCl4 injection) showed improvement in most parameters when compared with cirrhotic control group. It has been concluded that L-carnitine and coenzyme Q10 have a pronounced prophylactic effect against liver damage induced by halogenated alkanes.

Key words: Coenzyme Q10, halogenated alkanes, L-carnitine, lipid peroxides, LDH and reduced glutathione

INTRODUCTION

Liver is a complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified. It is responsible for synthesizing and
Liver is responsible for concentrating and metabolizing the majority of drugs and toxins\(^{(2)}\).

Liver function tests are valuable in detecting impairments of liver and impending hepatitis, drug toxicity, infiltrative lesions such as tumors and certain stages of cirrhosis\(^{(3)}\). The determination of ALT and AST is of a real value in distinguishing hepatocellular and obstructive jaundice. They are especially important in the early diagnosis of infective hepatitis\(^{(4)}\). It appeared that AST activity is more sensitive reflection of chronic hepatitis and that ALT is more readily elevated by acute hepatitis\(^{(5)}\).

GSH plays a key role in protection against tissue damage caused by oxidative stress\(^{(6)}\) and in regulation of the immune response\(^{(7)}\). The GSH is an important tool in the defense mechanism against reactive oxygen species, its depletion could be attributed to its consumption by the liberated free radicals\(^{(8)}\).

Carbon tetrachloride when administered is distributed throughout the whole body\(^{(9)}\), with highest concentrations in liver, brain, kidney, muscle, fat and blood. The parent compound is eliminated primarily in exhaled air, while minimal amounts are excreted in the feces and urine\(^{(9)}\). \(\text{CCl}_4\) intoxication, also, leads to hypomethylation of cellular components; in the case of RNA the outcome is thought to be inhibition of protein synthesis, in the case of phospholipids it plays a role in the inhibition of lipoprotein secretion. None of these processes is considered the ultimate cause of \(\text{CCl}_4\)-induced cell death; it is by cooperation that they achieve a fatal outcome, provided the toxicant acts in a high single dose, or over longer periods of time at low doses\(^{(10)}\).

L-carnitine is 4-trimethylamino-3-hydroxybutyrate. It is synthesized chiefly in the liver and kidneys from the essential amino acid L-lysine residues in certain proteins\(^{(11)}\). Chronic kidney disease and some forms of liver disease may be indications for L-carnitine supplementation. Preliminary work suggested that L-carnitine can reduce fat deposits in some fatty livers\(^{(12)}\). Carnitine deficiencies are occasionally associated with other diseases, such as diabetes and cirrhosis\(^{(13)}\).

Carnitine deficiency in humans is associated with myopathy\(^{(14)}\) and impaired fatty acid oxidation\(^{(15)}\). Coenzyme \(\text{CoQ}_{10}\) (CoQ\(_{10}\)) or ubiquinone is a vitamin-like substance that is synthesized in all tissues. CoQ\(_{10}\) is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III). The electron and proton transfer functions of the quinine ring are of fundamental importance to all life forms\(^{(16)}\).

The role of ubiquinone (CoQ\(_{10}\)) as a component of the mitochondrial respiratory chain, and an intracellular antioxidant has gained attention. In vitro study demonstrated that CoQ\(_{10}\) protected membrane phospholipids and serum LDL from lipid peroxidative stress\(^{(17)}\). In vivo study reported that CoQ\(_{10}\) reduced myocardial ischemia and reperfusion injury induced by oxidative stress.
through suppression of the formation of reactive oxygen species.\(^{(18)}\)

**MATERIALS & METHODS**

**Experimental animals**

Eighty male albino rats of 150-200g body weight (El-Nasr Pharmaceutical Company, Cairo, Egypt) were recruited for the present study. Rats were housed in metabolic cages at constant experimental condition of temperature of 25°C with 12 hours light/dark cycle. Animals were fed on rodent chow diet (ADWIC Co., Cairo, Egypt) and allowed free access of drinking water.

**Induction of Liver cirrhosis**

Liver cirrhosis was induced by s.c. injection of a single dose of CCl\(_4\) (1ml/kg)\(^{(19)}\). L-carnitine (50mg/kg/day) was given i.p for four successive days 24 hours before CCl\(_4\) injection\(^{(20)}\) and CoQ\(_{10}\) (200 mg/kg) was given as a single i.p dose 24 hours before CCl\(_4\) injection\(^{(21)}\). Animals were sacrificed 24 hours after CCl\(_4\) injection, blood samples were withdrawn and liver tissue samples were homogenized and stored at -20°C.

**Blood and Tissue sampling**

Blood samples were collected at the end of the study from fasting rats via retro-orbital plexus under diethyl ether anaesthesia and bleeding, processed for subsequent determinations of amino transferase enzymes (ALT & AST), total LDH activities, total protein and separation of LDH isoenzymes.

Livers were removed from sacrificed rats. Briefly the liver tissues were homogenized in hexane/propanol (3.2 v/v) and centrifuged. The extract was collected and washed with aqueous sodium sulfate. Supernatant was evaporated and the precipitate was weighed, dissolved in 10 ml hexane and stored at -20°C prior to analysis\(^{(22)}\). Lipid peroxides level (MDA), reduced glutathione, total LDH activities, total protein levels was determined and LDH isoenzymes were separated by gel electrophoresis.

Lipid peroxides level was determined according to the method of Ohkawa et al.\(^{(23)}\), reduced glutathione content was determined according to the method described by Moron et al.\(^{(24)}\). Serum and hepatic total protein levels were determined according to the method described by Bradford\(^{(25)}\). Amino transferases (ALT & AST) were determined according to the method described by Reitman and Frankel\(^{(26)}\), total LDH activities was determined according to the method described by Babson and Babson\(^{(27)}\) and separation of LDH isoenzymes by gel electrophoresis by the method of Dietz and Lubrano\(^{(28)}\).

**Statistical analysis**

Data were expressed as mean ± SE. Results were analyzed by student -t- test using InStat-3 program. \(P<0.05\) was considered statistically significant.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Groups</th>
<th>Cirrhotic Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (I)</td>
<td>L-Carnitine (II)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>29.32 ± 2.56</td>
<td>27.17 ± 3.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>71.18 ± 5.15</td>
<td>62.37 (*) ± 3.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Total Protein (mg/ml)</td>
<td>19.30 ± 0.42</td>
<td>17.32 ± 1.26</td>
</tr>
<tr>
<td>Serum LDH (µ/L)</td>
<td>81.42 ± 13.47</td>
<td>77.00 (*) ± 11.76</td>
</tr>
</tbody>
</table>

* Significantly different from group I at P < 0.05.
# Significantly different from group V at P < 0.05.
Table (2): Effect of L-Carnitine or/ and CoQ₁₀ treatment on hepatic MDA, GSH, total protein and LDH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Groups</th>
<th>Cirrhotic Groups</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (I)</td>
<td>L-Carnitine (II)</td>
<td>CoQ₁₀ (III)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Carnitine + CoQ₁₀ (IV)</td>
<td>Control (V)</td>
</tr>
<tr>
<td>MDA (nmol/mg )</td>
<td>2.57± 0.12</td>
<td>2.25 (*)± 0.18</td>
<td>2.34± 0.32</td>
</tr>
<tr>
<td>GSH (mg/g tissue)</td>
<td>3.93± 0.07</td>
<td>5.33 (*)± 0.88</td>
<td>6.19 (*)± 0.92</td>
</tr>
<tr>
<td>Hepatic Total Protein (mg/g tissue)</td>
<td>250.11± 23.58</td>
<td>258.28± 35.71</td>
<td>256.05± 8.83</td>
</tr>
<tr>
<td>Hepatic LDH (µ/L)</td>
<td>114.0± 1.05</td>
<td>88.9 (*)± 0.15</td>
<td>99.57 (*)± 4.43</td>
</tr>
</tbody>
</table>

* Significantly different from group I at P < 0.05.
# Significantly different from group V at P < 0.05.
Separation of serum LDH isoenzymes by gel electrophoresis:

Figure (2), tables (3) and (4) show the electrophoretic separation pattern of serum lactate dehydrogenase isoenzymes in normal and cirrhotic groups. It can be observed that CCl₄ injection resulted in a significant increase in serum levels of all LDH isoenzymes. Administration of L-carnitine alone significantly decreased serum LDH-1 and LDH-5; however, it increased serum LDH-2, LDH-3 and LDH-4 compared to CCl₄ control group. Administration of coenzyme Q₁₀ alone significantly decreased serum levels of all LDH isoenzymes compared to CCl₄ control group. On the other hand, administration of a combination of L-carnitine and coenzyme Q₁₀ significantly decreased serum LDH-1, LDH-2, LDH-3 and LDH-5; however, it increased serum LDH-4 compared to CCl₄ control group.

Fig. (1): Electrophoretic Profile of Serum Lactate Dehydrogenase Isoenzymes (LDH- Isoenzymes) of Male Rats.

Lane 1 : Normal Control Group
Lanes 2, 3 : L-Carnitine Group
Lane 4 : CoQ₁₀ Group
Lane 5 : Heart (Standard Group)
Lane 6 : L-Carnitine & CoQ₁₀ Group
Lane 7 : CCl₄ Control Group
Lane 8 : L-Carnitine & CCl₄ Group
Lanes 9, 10 : CoQ₁₀ & CCl₄ Group
Lanes 11, 12 : L-Carnitine, CoQ₁₀ & CCl₄ Group
Table (3): % serum LDH isoenzyme pattern in all groups

<table>
<thead>
<tr>
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<th>Cirrhotic Groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (I)</td>
<td>L-Carnitine (II)</td>
</tr>
<tr>
<td>% LDH-1</td>
<td>1.63 ± 0.102</td>
<td>2.25 ± 0.717</td>
</tr>
<tr>
<td>% LDH-2</td>
<td>2.10 ± 0.287</td>
<td>2.10 ± 0.0637</td>
</tr>
<tr>
<td>% LDH-3</td>
<td>4.15 ± 0.209</td>
<td>5.75 ± 0.288</td>
</tr>
<tr>
<td>% LDH-4</td>
<td>5.22 ± 0.204</td>
<td>8.61 ± 2.278</td>
</tr>
<tr>
<td>% LDH-5</td>
<td>86.88 ± 0.172</td>
<td>81.27 ± 3.296</td>
</tr>
</tbody>
</table>

* Significantly different from group I at P < 0.05
# Significantly different from group V at P < 0.05

Table (4): Activity of Serum LDH Isoenzymes (µ/L)

<table>
<thead>
<tr>
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<th>Cirrhotic Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (I)</td>
<td>L-Carnitine (II)</td>
</tr>
<tr>
<td>LDH-1</td>
<td>1.33 (*)</td>
<td>1.73 (*)</td>
</tr>
<tr>
<td>LDH-2</td>
<td>1.71 (*)</td>
<td>1.62 (*)</td>
</tr>
<tr>
<td>LDH-3</td>
<td>3.38 (*)</td>
<td>4.43 (*)</td>
</tr>
<tr>
<td>LDH-4</td>
<td>4.25 (*)</td>
<td>6.63 (*)</td>
</tr>
<tr>
<td>LDH-5</td>
<td>70.74 (*)</td>
<td>62.58 (*)</td>
</tr>
</tbody>
</table>

* Significantly different from group I at P < 0.05
# Significantly different from group V at P < 0.05
DISCUSSION

Cirrhosis is an irreversible result of various disorders that damage liver cells over time with no proven effective therapy. Eventually, damage becomes so extensive that the normal structure of the liver is distorted and its function is impaired\(^{(29)}\).

Reactive oxygen species (ROS) as free radicals can initiate lipid peroxidation and DNA damage leading to cell death, if the antioxidant system is impaired. Oxygen derived free radicals are continuously generated in the cells\(^{(30)}\). The production of ROS in the biological system results from the sequential univalent reduction of molecular oxygen\(^{(31)}\), leading to the formation of superoxide radical (\(O_2^-\)). Once formed, which undergoes a variety of chemical reactions yielding other ROS like hydroperoxyl radical (\(HO_2^-\)). Reaction between \(O_2^-\) and \(H_2O_2\) in the presence of certain transition metals such as iron can yield the potent oxidizing agent (\(OH^-\))\(^{(32)}\).

Oxidative stress has a role in liver injury, cirrhosis development and carcinogenesis\(^{(33)}\). Mitochondria is the main generator of superoxide in hepatocytes. The mechanism of superoxide production is linked to either the disorder in the operation of the ubiquinone (CoQ\(_{10}\)) cycle (complex III)\(^{(34)}\) and/or reducing equivalents which can not be transferred to \(O_2\) at the mitochondrial cytochrome-c oxidase, due to oxygen deficiency\(^{(35)}\).

Carbon tetrachloride injection is used to provide animal model of liver damage which is caused by formation of trichloromethylperoxyl radicals, initiating lipid peroxidation and resulting in fibrosis and cell necrosis\(^{(36)}\). The oxidative stress in rat liver and lipid peroxidation caused by administration of CCl\(_4\) has been reported\(^{(37,38)}\).

Carbon tetrachloride treated rats showed a significant increase of plasma activities of aminotransferases (ALT and AST), malondialdehyde (MDA) formation confirming other study\(^{(38,40,42)}\), a significant decrease in hepatic total protein, this result confirmed previous result\(^{(39)}\). Also, it showed decreased serum level of total protein confirming other result\(^{(43)}\) and hepatic reduced glutathione a result is in agreement with that of Allis et al.\(^{(38)}\). The earliest change in CCl\(_4\) hepatotoxicity is the blockage of lipoprotein secretion and the accumulation of lipids in the liver\(^{(44)}\).

In the present investigation, CCl\(_4\) injection produced a significant increase in serum total LDH activity finding confirming other studies\(^{(39,41)}\) and caused a significant increase in serum levels of all LDH isoenzymes. Also, there was a significant increase in hepatic LDH activity after injection of CCl\(_4\), in accordance with the result of Seeto et al.\(^{(45)}\), this is due to the increasing in LDH leakage from rat hepatocytes that may be attributed to CCl\(_4\)-induced dehalogenation in the liver endoplasmic reticulum. This process leads to trichlormethyl radical (CCl\(_3^-\)) formation and initiation of lipid peroxidation\(^{(46)}\).

In the present study, levels of MDA were shown to be significantly decreased on injection of L-carnitine in CCl\(_4\) induced hepatotoxicity. L-carnitine administration stimulates β-
oxidation of fatty acids and reduces the esterification of triacylglycerol\(^{47}\) and its level in the liver tissue was found to be low in the case of cirrhosis\(^{48}\). L-carnitine may lead to the inhibition of lipid peroxidation by enhancing antioxidant capacity\(^{49}\).

The CoQ\(_{10}\) was accompanied by a marked reduction of lipid peroxides level and normalization of reduced glutathione levels in liver tissue of rats injected with CCl\(_4\). These results confirm the beneficial antioxidant activity of ubiquinone\(^{50,18,51}\). Administration of ubiquinone significantly prevented CCl\(_4\) induced oxidative stress and lipid peroxidation in rat liver\(^{37,38}\) because the coenzyme Q\(_{10}\) increases the activity of the electron transport chain both in vitro and in vivo\(^{42}\), beside its antioxidant effects\(^{53,54}\).

Administration of L-carnitine or/and coenzyme Q\(_{10}\) (prior to CCl\(_4\) injection) significantly increased hepatic and serum total protein, hepatic reduced glutathione content and significantly decreased serum ALT and AST activities compared to CCl\(_4\) control group. Marked improvement has been achieved by the combination of L-carnitine and coenzyme Q\(_{10}\) in agreement with the result of Wang et al\(^{42}\).

Administration of L-carnitine or/and coenzyme Q\(_{10}\) (prior to CCl\(_4\) injection) significantly decreased hepatic and serum total LDH activity compared to CCl\(_4\) control group in harmony with other studies\(^{39,42}\).

LDH-1 isoenzyme is maximally active at low concentration of pyruvate and inhibited by excess pyruvate, while LDH-5 isoenzyme maintains its activity at high pyruvate concentration\(^{55}\). The electrophoretic separation pattern of serum lactate dehydrogenase isoenzymes in normal and cirrhotic groups showed that CCl\(_4\) injection resulted in a significant increase in serum levels of all LDH isoenzymes. The treatment with L-carnitine resulted in a significant increase in isoenzyme 2, 3 and 4; and a significant decrease in isoenzyme 1 and 5. Treatment with coenzyme Q\(_{10}\) resulted in a significant decrease in all isoenzymes. Treatment with a combination of L-carnitine and coenzyme Q\(_{10}\) resulted in a significant increase in isoenzyme 4 and a significant decrease in isoenzyme 1, 2, 3 and 5.

**Conclusion:** From the previous results, it could be concluded that L-carnitine and coenzyme Q\(_{10}\) have a pronounced prophylactic effect against liver damage induced by halogenated alkanes and the combination of L-carnitine and CoQ\(_{10}\).

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32. **Evans P.H.**


دور بيتا- هيروكسي- جاما- ثلاثي ميثيل الأمونيوم بيوتيرات و ويبيكينون
في تأثير الأكسدة للألياف الهالوجينية على الكبد

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أسماء مصطفى, أحمد بيومي
قسم الكيمياء الحيوية - كلية الصيدلة - جامعة المنهاج وقسم الكيمياء الطبية -
المركز القومي للبحوث، الدقي، مصر

الهدف من هذا البحث: تم تقديم تعاملات التأثير الضار للألياف الهالوجينية (رابع كلورد الكربون) على
خلال الكبد وتقديم دور كل من د. كارتيناج ومساعد الإنزيم كيوب 10 في الوقاية من هذا التأثير الضار.
أجريت هذه الدراسة على 10 ذكور القد اثنان البالغة حيث قسمت القدان إلى ثلاثة مجموعات (كل
مجموعة 10 جذوران) كالتالي: مجموعة ضاربة، مجموعات تحت برمولات كارتيناج، مجموعة
حقن تحت برمولات القد صفر، و مجموعات تحت برمولات القد 10. و تم استخدم التأثير
الكبد (محمولة ضاربة، مجموعات تحت برمولات كارتيناج، مجموعة حقن تحت برمولات القد صفر،
ومجموعات تحت برمولات القد 10) و تأثير القد صفر و مجموعات تحت برمولات القد صفر،
لم يتم ساعة (1). بينما تم الحقن البيريديتل على د. كارتيناج (50 مجم/كم/يوم) لمدة 1 أسابيع متتالية وجرعة
واحدة من كيوب كيوب 10 (000 مجم/كم) قبل الحقن رابع كلورد الكربون ب 24 ساعة.
ومت ذهب جميع القدان بعد حقن رابع كلورد الكربون ب 24 ساعة، وسحبت عينات الدم لفصل مصل الدم,
и وفصل الكبد وعمل خليط محتوي منه وتحقيقها لحين الاستعمال. تم قياس كل من المحتوى الكبدی
للخلايا التائية المصغرة، شرط البريمي مللي الكبدية للالادوات نتيجة مفصل القد، المحتوى الكبدية للألياف الهالوجينية
للدوب، مجموعات البرزين الكبدية في مصل الدم، مجموعات البرزين الكبدية في مصل الدم،
ومستوى الطاقة لإنزيم اللالادوات ديديرجيزيز في مصل الدم
و مستوى النظام لإنزيم اللالادوات ديديرجيزيز في مصل الدم.
و كنتيجة الدراسة أن حقن رابع كلورد الكربون يؤدي إلى تخافش جزئي إحماسية في المحتوى
الكبدية لإنزيمات المختزل، البرزين الكبدية في الكبد، و مصل الدم، كما يؤدي إلى إرفاق نفسية في
نشاط البريمي مللي الكبدية للألياف الهالوجينية للدوب، مستوى إنزيم
الالادوات ديديرجيزيز في مصل الدم والكبد يؤدي إلى إرفاق جزئي إحماسية في مستويات النظائر المختلفة
لإنزيم الانزيمات ديديرجيزيز في مصل الدم. ومن ناحية أخرى، فقد ثبت أن حقن البيريديتل على د. كارتيناج أو
مساعد الكبد كيوب 10 و كيوب كيوب 10 و كيوب كيوب 10 و كيوب كيوب 10 و كيوب كيوب 10 و كيوب كيوب 10 و كيوب كيوب 10 و
التضامن باللائحة المختلفة لل帚اء التي تتم تضامنها باللائحة المختلفة لل帚اء.
كما فقد أوضحت هذه الدراسة الدور الوقائي لل كارتيناج ومساعد الكبد كيوب 10، 10 كمضادات للألياف الهالوجينية.
خلايا الكبد من التلف الناتج عن الألياف الهالوجينية.