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Effect of Biosynthesized L-carnitine from *Pleurotus ostreatus* against Acute Paracetamol Hepatotoxicity and Nephrotoxicity in Mice

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ABSTRACT

L-carnitine is a cofactor in the transfer of long-chain fatty acid allowing the β-oxidation of fatty acid in the mitochondria. It is also a known antioxidant with protective effects against lipid peroxidation. This study aimed to investigate the effect of the biosyenthesized L-carnitine from mushroom *Pleurotus ostreatus* against paracetamol (AA) induced hepatotoxicity and nepherotoxicity where mitochondrial dysfunction and oxidative stress are thought to be involved in AA toxicity. Forty Swiss albino male adult mice were divided into five groups. Mice were dosed with single-AA injection (500 mg/kg via the intra peritoneal route) with or without L-carnitine (500 mg/kg for 10 days starting 10 days before and after AA injection via intra peritoneal route) AA increased serum AST, ALT, serum creatinine and BUN levels significantly. Administration of biosynthesized L-carnitine from *Pleurotus ostreatus* significantly reduced AA-induced elevations in AST, ALT, serum createnine and BUN levels. Also histopathological examination of liver and kidney showed great improvement of AA induced necrosis and hemorrhage. These results suggested that AA results in oxidative damage in the liver and kidney with an acute effect and L-carnitine from mushroom *Pleurotus ostreatus* pharmacologically active and has a prominent therapeutic and protective effect against acute AA toxicity and may be of therapeutic value in the treatment and prevention of AA-induced hepatotoxicity and nephrotoxiciy.

Keywords: L-carnitine hepatoprotection, nephroprotection, acetaminophen Toxicity.

1. INTRODUCTION

L-carnitine is a γ-three 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. By combination with carnitine to form acylcarnitine, acyl groups could be transferred from cytosolic coenzyme A on the outer surface of the mitochondrion membrane, then to the inner surface by exchange with free carnitine using an antiport mechanism. The acyl groups are then transferred from carnitine to coenzyme A within the mitochondrion [1]. Carnitine is also associated with buffering of excess acyl-Co A, which is potentially toxic to the cells, and it was reported that L-Carnitine has a protective effect on lipid peroxidation by reducing the formation of hydrogen peroxide [2,3]. L-carnitine could also improve antioxidant status in rats and showed free radical scavenging activity as well [4,5].

Due to the important role of L-carnitine in human body it was implicated in treatment of many diseases and drug toxicities and there were an urgent demand for L-carnitine biosynthesis which was produced in the past by chemical methods which were complex and very high cost methods. Recently it was biosynthesized by using microorganisms such as *Pleurotus ostreatus* mushroom. This study aimed to investigate if the biosynthesized L-carnitine from mushroom pharmacologically active or not against paracetamol toxicity. As Paracetamol or Acetaminophen (AA) is widely prescribed as analgesic and antipyretic drug in the clinic and is sold in numerous

over-the-counter preparations as a single compound or in combination with other medications [6,7]. It is well tolerated in prescribed doses [8] and because of its wide availability there is a much higher risk for over dose [9].

Paracetamol toxicity is one of the most common causes of poisoning worldwide. It is involved in 30 and 40% of acute presentation with poisoning cases in hospitals. The toxicity of paracetamol is well understood but there is a great ignorance among public. The toxic dose of paracetamol is highly variable. In general the recommended maximum daily dose for healthy adults is 4 grams. Higher doses lead to increasing risk of toxicity. In adults, single doses above 10 grams or 200 mg/kg of body weight have a reasonable likelihood of causing toxicity [10].

Toxicity can also occur when multiple smaller doses within 24 hours exceeds these levels in adults, a dose of 6 grams a day over the preceding 48 hours could potentially lead to toxicity [11] .In rare individuals, paracetamol toxicity can result from normal use. This may be due to individual idiosyncratic differences in the expression and activity of certain enzymes in one of the metabolic pathway that handle paracetamol [12].

Paracetamol overdose lead to severe hepatotoxicity and nephrotoxicity which may lead to death, and in some cases, renal disease has occurred in the absence of significant hepatotoxicity. Studies are go for searching on a protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body [13].

Aim of the work to study if the biosynthesized L-carnitine from mushroom *pleurotus ostreatus* pharmacologically active or not against paracetamol induced hepatotoxicity and nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Chemicals and equipments

1. Perfalgan (1 g/100 ml infusion) (Bristol Myer Squib, Egypt).
2. L-carnitine (chemical) (Lonza, Switzerland).
3. Kits for estimation of ALT,AST,serum creatinine and BUN(Biomerièux, France).
4. Spectrophotometer (Shimadzu/Double beam spectro-photometer U.V.150, Germany).
5. Centrifuge (Narco- Biosystem, U.K.).

2.2 Experimental design

Fourteen week old adult male Swiss Albino mice weighing 20-25 grams each were used in this investigation. Mice were maintained in an animal facility under standard laboratory conditions for 2 weeks prior to experiments. The mice were housed at 23-25 C and in daily dark/light cycle. Mice were caged (8 per cage) in fully ventilated cages and were provided with water and standard chow ad libitum. All experiments were carried out in accordance with protocols approved by the local experimental animal's ethics committee Mice were divided into the following groups (8 mice/group):

Group 1: Control group

Mice in this group served as normal controls and injected (single dose) with distilled water via the intra-peritoneal route. Mice were scarified 24 hours after injection with distilled water and blood was collected via cardiac puncture and tissues were prepared for histopathological studies.

Group 2: Control group

In this group hepatotoxicity and renal toxicity were induced by intra-peritoneal injection of perfalgan (paracetamol, 1 g/100 ml infusion) in a dose of 500 mg/kg/single dose.

Group 3: Chemical L-carnitine treated group (CLcar-T)

In this group hepatotoxicity and renal toxicity were induced by intra-peritoneal injection of perfalgan (paracetamol, 1 g/100 ml infusion) in a dose of 500 mg/kg/single dose. Mice were then injected with chemical L-carnitine in a dose of 500 mg/kg/day via intra-peritoneal route for 10 days. Mice were scarified 24 hours after 10th chemical L-carnitine doses. And this group used as positive control.

Group 4: Biosynthesized L-carnitine treated group (BLcar-T)

In this group hepatotoxicity and renal toxicity were induced by intra-peritoneal injection of perfalgan(paracetamol, 1 g/100 ml infusion)in a dose of 500 mg/kg/single dose. Mice were then injected with biosynthesized L-carnitine in a dose of 500 mg/kg/day via intra-peritoneal route for 10 days. Mice were scarified 24 hours after 10th biosynthesized L-carnitine dose.

Group 5: Biosynthesized L-carnitine pretreated group (BLcar-P)

Mice In this group were injected with biosynthesized L-carnitine in a dose of 500 mg/kg/day via intra-peritoneal route for 10 days before the induction of paracetamol induced hepatotoxicity and renal toxicity. Following biosynthesized L-carnitine pretreatment, mice were injected with perfalgan (paracetamol, 1g/100 ml infusion) via intra-peritoneal route in a dose of 500

mg/kg/single dose. Mice were scarified 24 hours after paracetamol injection.

2.3 Blood sampling

Blood samples were collected via cardiac puncture 24 hours following water injection of control (group 1), paracetamol injection (groups 2 and 5) or final L-carnitine dose (groups 3 and 4) using a fine heparinized capillary tube.One and half millimeter of blood were collected in a clean graduated centrifugal tube and left for clotting at room temperature in water bath for 15 minutes. Serum samples were then obtained by centrifugation for 10 minutes at 4000 rpm. The supernatant serum was collected in a dry tube and kept at - 20C for estimation of alaninie aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and blood urea nitrogen (BUN). Using commercially available kits (Biomerièux, France), and according to the manufacturer protocols.

2.4 Estimation of serum markers

Levels of alaninie aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine and blood urea nitrogen were estimated using commercially available kits (Biomerièux, France), and according to the manufacturer protocols.

2.5 Histopathological examinations

Samples of liver and kidney tissues were stained with haematoxylin and eosin stain for general architecture of the studied organ.

3. RESULT AND DISCUSSION

3.1 Statistical analysis

Results were expressed as mean ± of standard error (MSE). Repeated-measures Analysis of Variances (ANOVA) was used for statistical analysis of the different groups using (spss) software and the probability of chance "p values". As regards the probability ($p < 0.05$= significant).

3.2 Treatment with biosynthesized L- carnitine from mushroom compared with chemical L- carnitine against Paracetamol hepatotoxicity

Oxidative stress caused by AA results in the release of LDH, a marker of cell damage, and the release of several soluble products, including ALT, and AST The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage as a consensus defined the liver injury as an increase of more than twice the upper limit of the normal range (ULN) in the levels of serum alanina aminotransferase (ALT) or AST and alkaline phosphatase (AL P) according to type of injury [14].

The mice treated with an overdose of AA developed significant hepatic damage, which was observed by a substantial increase in the concentration of serum enzymes (ALTand AST). The obtained results of serum ALT and AST activity in control mice (Group I) showed the least activity among the experimented mice as it was (44.24 and38.22) .On the contrary the highest ALT and AST activity was recorded in sera of animals treated with AA (Group II) which was (1003.12 and 990.75) as shown in Table 1. This indicates severe hepatic injury as (ALT and AST) elevated more than twice the (ULN).

Table No 1. Serum parameters for all groups

Serum parameter	Control group	paracetamol group	c-L-car t group	B-L-car Tgroup	B-L-car p group
ALT IU/L	44.25± 4.8	1003.12± 20.53	848.6± 18.38	780.9± 17.9	815.33± 18.6
AST IU/L	38.22± 4.2	990.75± 19.7	834.17± 18.6	765.25± 17.3	807.2± 18.3
creatinine g/dl	4.6± 1.1	15.7±2.8	11.2± 2.1	6.3± 1.8	10.8± 1.9
BUN mg/dl	173.3± 8.5	593.2± 22.7	490± 20.3	275.88± 11.4	410± 17.3

Different superscripts in the same line indicate significant differences ($p<0.05$).

Administration of biosynthesized L- carnitine after AA treatment resulted in a significant reduction of AA-induced elevation of ALT and AST as it were(780.9 and 765.25) and appears to be protective in reducing the injurious effect of AA as shown in table 1 compared with chemical L- carnitine results which were (848.6 and 834.17). These results indicate that treatment with biosynthesized L- carnitine from mushroom reduced AA induced hepatotoxicity as the biochemical parameters and hepatic function tests were restored to control levels.

In addition histopathological examination of liver tissue after paracetamol toxic dose administration showed massive diffuse degenerative changes with few normal hepatocytes located around central vein the degenerated hepatocytes had vacuolated feathery cytoplasm. Inset showed closer view for both normal and degenerated hepatocytes compared with control group (Fig.1) as in

Figure 2. After administration of biosynthesized L- carnitine Liver biopsy specimen showed normal architecture and cellular components with few focal feathery degenerative changes and lymphoid aggregates in (25%). While (75%) showed no considerable pathological changes as shown in figure 4 compared with chemical L- carnitine as the hepatic degenerative changes were observed in (60%) that ranging from few to severe ones as shown in Figure 3.

Also pretreatment with biosynthesized L- carnitine before administration of paracetamol toxic dose lead to significant decrease of the induced elevation of ALT and AST which were (815.33and807.2) as shown in table 1. In addition histopathological examination of Liver biopsy specimen showed massive diffuse degenerative changes in the hepatocytes with lymphocytic aggregates in (50%). While (50%) showed no considerable pathological changes as in figures 4, 5.These results reflect the role of L-carnitine as direct antioxidant which prevent the toxic effect of (AA) and these results are in line with results reported [6, 15, 16].

3.3 Treatment with biosyenthesized L- carnitine from mushroom compared with chemical L- carnitine against Paracetamol nephrotoxicity

Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine dearrangements.Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man [17].

Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine [18] .In the present study, administration of a toxic dose of (AA) showed a significant increase in the serum creatinine and urea concentrations in the Group 2 (AA group) as it were (15.7 and 593.22) when compared to the control group (Group 1) which were (4.6and 173.3). Moreover administration of biosynthesized L- carnitine via ip route significantly reduce the serum creatinine and urea elevation as it were (6.3and 275.88) as in Table 1. Compared with chemical L-carnitine as its results were (11.2and 490) and these results are in harmony with results reported [6,15, 16].

In this study, AA induced nephrotoxicity showed a significant increase in the serum creatinine and urea concentrations in the Group 2 (AA group) as it were (15.7 and 593.22) when compared to the control group (Group 1) which were (4.6and 173.3). Moreover administration of biosynthesized L- carnitine via ip route significantly reduce the serum creatinine and urea elevation as it were (6.3and 275.88) as in table 1. compared with chemical L- carnitine as its results were (11.2and 490) and these results are in harmony with results reported [6, 15, 16]. In addition pretreatment with biosynthesized L- carnitine also lead to improvement in both serum creatinine and urea elevation as it became (10.8and 410) as in table 1.

The biochemical results were also confirmed by the histopathological findings of renal biopsy specimen showed focal areas of coagulative necrosis and hemorrhage in (25%) of the studied mice. Renal tissue showed necrotic area demonstrating ghosts of renal tubules, casts and lymphocytic infiltrates. The necrotic area was sharply separated from the adjacent normal renal tissue in paracetamol group as in figure 7 compared with control in figure 6.

In treated and pretreated groups with biosynthesized L-carnitine from mushroom *Pleurotus ostreatus*, renal biopsy specimen showed normal glomeruli and tubules in all studied animals as in figure9 and figure 10 which in harmony with the results of chemical L- carnitine figure 8.This results confirm that Antioxidants like L-carnitine have been found to offer protection against AA-induced liver and renal damage. These results come in line with [15&19]. The mechanism associated with the protective effect of L-carnitine could be also due to direct antioxidant effect of L-carnitine. Similarly, the antioxidant effect of L-carnitine was effectively utilized to prevent the toxic effect of several chemicals. For example, cisplatin-induced nephrotoxicity where oxidative stress and lipid peroxidation are thought to play a major role in the pathophysiology of nephrotoxicity,administration of L-carnitine in Sprague–Dawley rats normalized kidney function. In addition-carnitine attenuated the increased MDA and reduced GSH levels [20]. Other than nephrotoxicity,L-carnitine was shown to prevent ethanol-induced lesions in the gastric mucosa and protected against lipid peroxidation as well as normalized GSH of gastric mucosa in rats [21]. Methamphetamine neurotoxicity, mediated by peroxynitrite radicals, was protected by L-carnitine [22]. In addition, it was reported that mitochondrial proteins could be the target for AA toxicity leading to the loss of energy production and cellular ion control [23].

The action of L-carnitine in mitochondrial energy production is to facilitate the transfer of long-chain fatty acids from cytosol to mitochondria, thereby playing an important role in the production of ATP [1]. Indeed, L-carnitine was shown to increase ATP production in the myocardium in cisplatin-induced cardiomyopathy [24] and improve the oxidative stress caused by (AA).

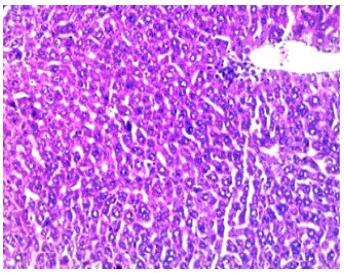

Fig.1Control liver

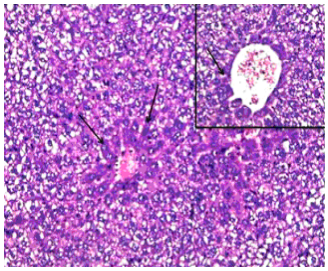

Fig.2 Paracetamol liver

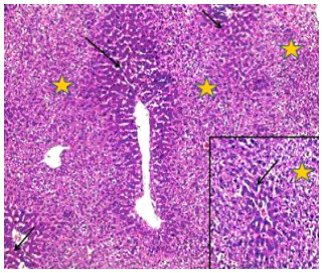

Fig.3 Chemical L-car liver

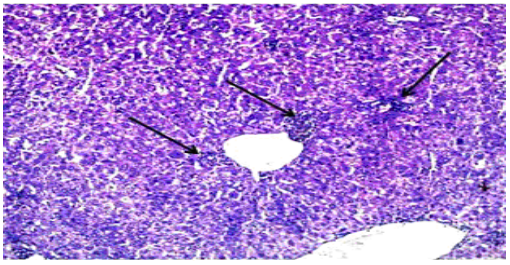

Fig.4 B-L-car Tgroup liver

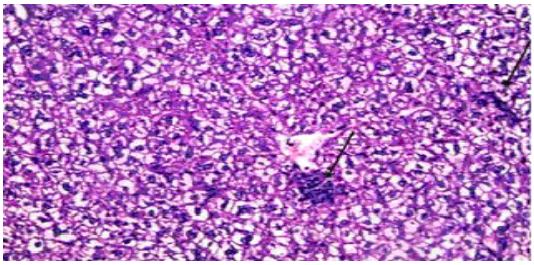

Fig.5 B-L-car p group liver

Histopathology of liver (Fig 1 to Fig 5)

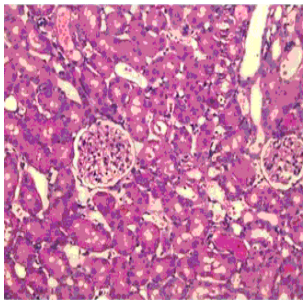

Fig.6 Control kidney

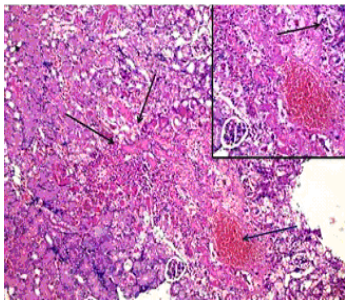

Fig.7 Paracetamol kideny

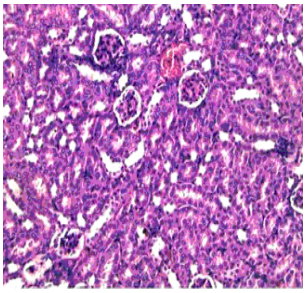

Fig.8 Chemical L-car kidney

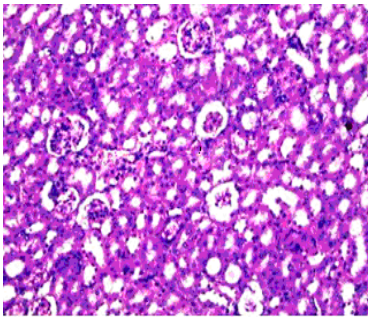

Fig.9 B-L-car Tgroup kidney

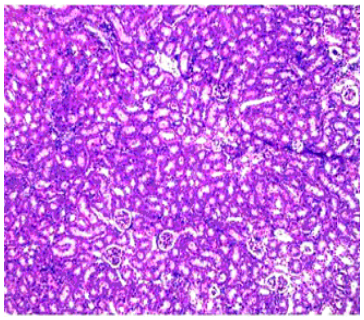

Fig.10 B-L-car p group kidney

Histopathology of kidney (Fig 6 to Fig 10)

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