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Study the Nephro-Protective Effects of L Carnitine on Rats

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ABSTRACT

Carnitine is biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. Methods: 40 albino rats were enrolled in this study, and they were divided into four groups, group I (10 rats) acts as control, and group II (10 rats) was treated with Gentamicin 80mg/kg. The group III (10 rats) was treated with L carnitine, and the group IV was treated with l carnitine and gentamicin. Results: showed that L carnitine has statistically significant nephro-protective effects. The nephro-protective effects were assessed by: 1-histopathological studies. 2- Measuring serum levels of BUN, and creatinnine. In group treated with gentamicin and L-carnitine, the mean of serum levels of urea was 65.6±0.7 mg/dl. This level showed a significant decrease as compared to that in gentamicin treated group (92.3±1.2 mg/dl) P value < 0.05. Conclusions: L carnitine has nephro-protective effects.

Key words: Nephro-protective, L carnitine , Gentamicin

Introduction

Carnitine is biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. It is often sold as a nutritional supplement (Steiber *et al*., 2004). In animals, Carnitine is biosynthesized primarily in the liver and kidneys from the amino acids lysine (viatrimethyllysine) or methionine. Vitamin C (ascorbic acid) is essential for the synthesis of Carnitine. During growth or pregnancy, the requirement of Carnitine might exceed its natural production (Cederblad *et al*., 2008). Carnitine `s primary mechanism of action is apparently attributable to its role as a cofactor in the transformation of free long-chain fatty acids into acylcarnitines for subsequent transport into the mitochondrial matrix (Jogl *et al*., 2004). Carnitine is involved in the metabolism of ketones for energy (Fukao, *et al*., 2004) and the conversion of branched-chain amino acids – valine, leucine, and isoleucine – into energy (Platell *et al*., 2000).

Although L-carnitine is supplied exogenously as a component of the diet and can also be synthesized endogenously, evidence suggests both primary and secondary deficiencies can occur. Carnitine deficiency may be acquired or a result of inborn errors of metabolism (Stanley, 2004). Pre-term infants are at risk for developing a carnitine deficiency due to impaired synthesis and insufficient renal tubular resorption (Evangeliou and Vlassopoulos, 2003). Deficiency can result in cardiomyopathy, congestive heart failure, encephalopathy, hepatomegaly, impaired growth and development in infants, and neuromuscular disorders. Primary carnitine deficiency, although rare, is characterized by low levels of carnitine in plasma, red blood cell, and tissue. Generally deficiency of carnitine is associated with symptoms such as muscle fatigue, cramps, and myoglobinemia following exercise. Additional symptoms of chronic carnitine deficiency can include hypoglycemia, progressive myasthenia, hypotonia, or lethargy. Secondary carnitine deficiency is not rare and is most commonly associated during dialysis in chronic renal failure (Lynch *et al*., 2008; Rathod *et al*., 2006). Although it can also be induced by intestinal resection, severe infection, and liver *disease* (Cruciani *et al*., 2004).

Other conditions associated with a carnitine deficiency include cancer, diabetes, alzheimer's disease, and heart failure. (Evangeliou and Vlassopoulos, 2003).

L-carnitine has been extensively studied for patients in renal failure. Supplementation, either orally or intravenously, interferes with some of the disorders associated with dialysis, including anemia as a result of chronic renal diseases CRD, cardiac dysfunction, insulin resistance, lipid abnormalities, and oxidative stress (Matsumoto *et al*., 2001).

Materials and Methods

Animals:-

Male albino rats weighing (130±20 gm) were obtained from the animal house of Al-Nile Pharmaceutical Company. They were housed in stainless steel cages under a 12 hours light / dark cycle at room temperature.

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Animals were kept under the same condltion with regard food and water all over the period of this study.Each individual animal was weighed before start of therapy and was clearly marked by gentian violet to indicate its weight. The doses of drugs were accurately calculated according to the weight of each animal.

Apparatuses:

1. Centrifuge.VEB MLW Zentrifugenbav engelsdorf type T52.1.
2. Spectrophotometer "Shimadzu, UV-visible recording spectrophotometer UV-160, Shimadzu Corporation Kyoto-Japan".

Drugs and chemicals:

In this study, the following drugs and chemicals were used:
1-Gentamicin sulphate (Epigent) was obtained from: E.I.P.I.Co.
2-L-Carnitine was obtained from: AIkan Pharma, Egypt.
3-Urea and creatinine kits.

Induction of Nephropathy in Rats:

The rats received ordinary rat diet for 6 weeks and were given gentamicin injection in a dose of 80 mg/kg sub-cutaneausly once daily (Joel *et al*., 2002) during the last 8 consecutive days. We use the previous two models for induction of nephropathy because they are simple and available.

Study design:

Animals were randomized after estimation of basal blood urea and serum creatinine into four groups as follows:

Group I

10 rats received ordinary rat diet for 6 weeks and served as control group.

Group II

10 rats received ordinary rat diet for 6 weeks and were given gentamicin injection in a dose of 80 mg/kg sub-cutaneausly once daily (Joel *et al*., 2002) during the last 8 consecutive days.

Group III

10 rats received ordinary rat diet concomitant with L-carnitine orally by gastric tube (200 mg/kg/day) (Dayanandan *et al*., 2001) for 6 weeks

Group IV

10 rats received ordinary rat diet concomitant with L-carnitine orally by gastric tube (200 mg/kg/day) (Dayanandan *et al*., 2001) for 6 weeks and gentamicin injections (80 mg/kg/day) S.C. in the last 8 consecutive days.

Biochemical analysis and technique:

1. Serum urea (measured in mg/dl).
2. Serum creatinine (measured in mg/dl).

Determination of serum urea:

Blood urea levels were determined following urease modifled berthelot reaction (Fawcett and Scott, 1960) using kit supplied by Biomerieux- France.

Reagents:

1. Reagent 1 (standard): urea 50 mg/dl.
2. Reagent 2 (enzyme): urease
3. Reagent 3 (color reagent): phosphate buffer (PH == 0.8), sodium salicylate, sodium nitroprusside and ethylenediaminotetra-acetic acid (EDTA).
4. Reagent 4 (alkaline reagent): sodium carbonate and sodium hypochloride
5. Working reagent: one bottel of reagent 3 was reconstituted with one vial of reagent 2 and shacked gently.

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Procedure:

1. 1 ml of working solution was pipetted into 3 test tubes (test, blank and standard)
2. 10 ul of tested serum sample and 10 ul of standard solution were added to their corresponding tubes.
3. The tubes were mixed and incubated at 37C° for 3 minutes.
4. 200ul of reagent 4 was added to each ofthe three tubes.
5. The tubes were mixed and incubated at 37C° for 5 minutes
6. The absorbance was read using a spectrophotometer at wave length of 580 nm and setting the blank at zero.

Calculation:

$$Serum\ urea = \frac{A\ bsorbance\ A\ sample}{A\ bsorbance\ A\ standard} \quad X\ 50.\ mg/dl.$$

Determination of serum creatinine

Serum creatinine was determined by kinetic measurements according to the methods of (Bartels, 1971) using kit supplied by Human-Germany.

Principle:

The complex formed by creatinine and picric acid in an alkaline medium is measured for two minute

Reagents:

1. Reagent 1 (standard): creatmine 2 mg/dl.
2. Reagent 2 (colour reagent): picric acid.
3. Reagent 3 (alkaline reagent): sodium hydroxide diluted with distilled water in the ratio 1:4.
4. Working reagent: one volume of reagent 2 was mixed with one volume of reagent 3.

Procedure:

1. 1 ml of working solution was pipetted into 2 cuvettes (test and standard).
2. 100 ul of tested serum sample and 100 ul of standard solution were added to their corresponding cuvettes.
3. The cuvettes were mixed and stopwatch was started immediately.
4. The absorbance was read using a spectrophotometer at wave length of 492 nm between t= 20 sec and t= 80 sec where the zero adjustment was the air.

Caculation:

Serum creatinme (mg/dl)=$\frac{\Delta A\ sample}{\Delta A\ standard}$ X 2

$\Delta A = A_2 - A_1$

A_2: absorbance 60 seconds after first measurement A_1.
A_1: absorbance at 20 seconds.

Collection of kidney for histopathological examination:

At the end of 6th week after drug or vehicles administration the animals were sacrificed and the kidneys were removed for histological and ultra structure examination. One kidney was cut longitudinally; one half was fixed in 10% buffered formalin and embedded in paraffin. Sections of 5-mm thickness were cut and stained with Mayer's hematoxyline and eosin (for examination of cell structure) and examined by light microscope (Kelly *et al*., 2000).

Statistical analysis of results:

The variability of results was expressed as the mean ± standard deviation (X ± SD). Statistical analysis of the difference between groups was performed by using Statistical Package for the Social Sciences (SPSS) version 20, using one-way analysis of variance (ANOVA), and paired T test. Charts were done using Excel

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program, Microsoft Office XP 2007.

Degree of significance:

P > 0.05 = insignificant difference**.**
P < 0.05 = significant difference**.**

Results:

Serum urea:

The mean of serum urea levels of the control group was 28.2±1.5mg/dl, and in gentamicin treated group was 92.3±1.2mg/dl. This level showed a significant increase compared to the control group (P<0.05).

Our study found that in L-carnitine treated group the mean serum levels of urea was 24.6±1.0mg/dl. This level showed no significant change compared to control group (28.2±1.5mg/dl), whereas, in group treated with gentamicin and L-carnitine, the mean of serum levels of urea was 65.6±0.7mg/dl. This level showed a significant decrease as compared to that in gentamicin treated group (92.3±1.2 mg/dl) by using paired T test, the P value < 0.05.

Serum creatinine:

Our results expressed as mean ± SD (Table (1), Figure (2) show that in the control group, the mean of serum levels of creatinine was 0.62±0.05 mg/dl, and in group treated with gentamicin ,the mean of serum levels of creatinine was 2.7±0.09 mg/dl. This level showed a significant increase compared to control group(P<0.05).

Regarding results for L-carnitine treated group, the mean of serum levels of creatinine was 0.6±0.06 mg/dl. This value showed no significant change compared to that results for control group. The results regarding to group treated with gentamicin and L-carnitine, the mean of serum creatinine was 1.2±0.2 mg/dl, this value showed a statistically significant decrease compared to gentamicin treated group (P<0.05). our results proved that Lcarnitine has nephroprotective effects

Table 1: The mean plasma serum urea and creatinine (mg/dL) measured after oral administration of L-carnitine in a dose of 200 mg/kg/day in absence and presence of gentamicin in rats treated groups.

Treatments	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Group I served as a control	28.2±1.5	0.64±0.07
Group II treated with Gentamicin only	92.3±1.2*	3.12±0.37*
Group III treated with L carnitine only	24.6±1.0	0.62±0.04
Group IV treated with L carnitine and Gentamicin	65.6±0.7▪	2.48±0.37▪

*All data are mean ± Standard deviation (SD). *Significant increase from normal control group, ▪Significant decrease from gentamicin treated group.*

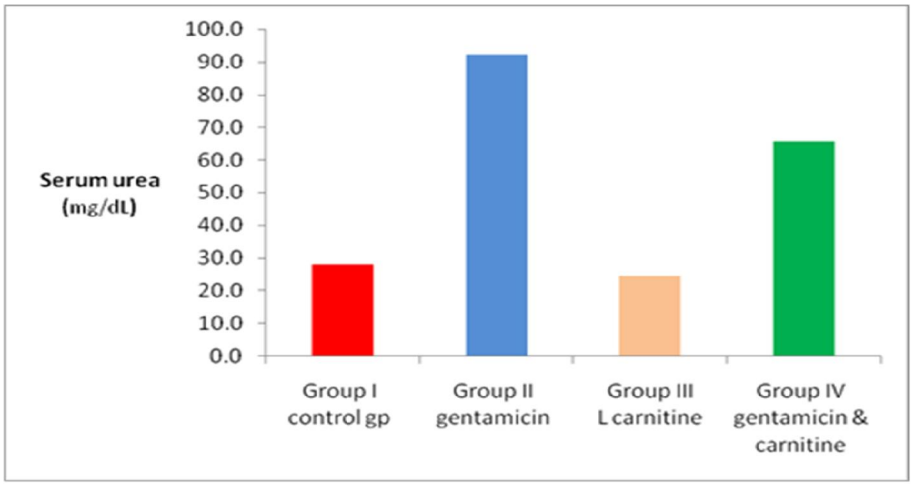

Fig. 1: The mean plasma urea (mg/dl) observed after adminstration of different treatments to groups: control gp, gentamicin gp., L carntine gp, and L carnitine with gentamicin gp (each group consists of 10 rats).

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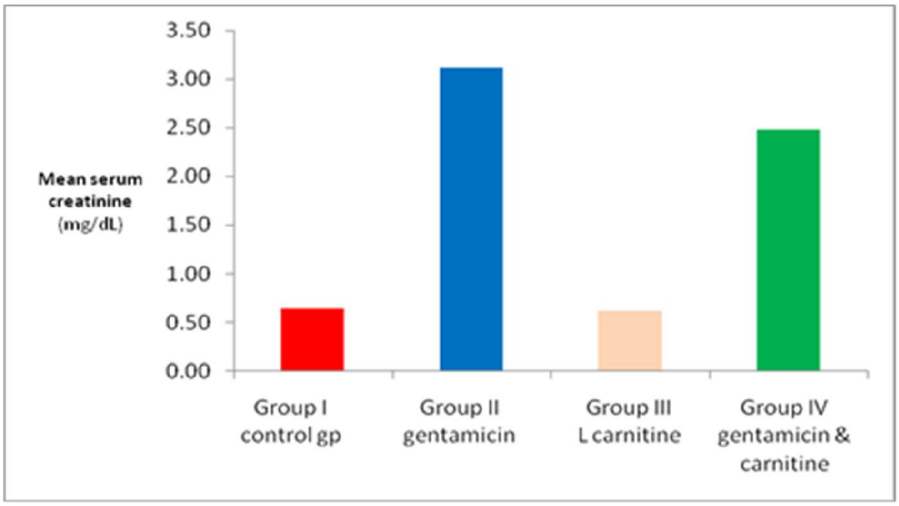

Fig. 2: The mean plasma creatinine (mg/dl) observed after adminstration of different treatments to groups: control gp, gentamicin gp., L carntine gp, and L carnitine with gentamicin gp (each group consists of 10 rats).

Histopathology

In the control group, section of rat kidney shows normal appearance of glomeruli and tubules, (figure 3). In gentamicin treated group, section of rat kidney shows dilatation of the tubules with drop out of some epithelial lining (picture of tubular necrosis), (figure 4).

Rats treated with L-carnitine, animals show normal appearance of glomeruli and tubules, wheras, the rats treated with gentamicin and L-carnitine, show dilatation of the tubules with drop out of some epithelial lining and mild atrophy of some glomeruli.

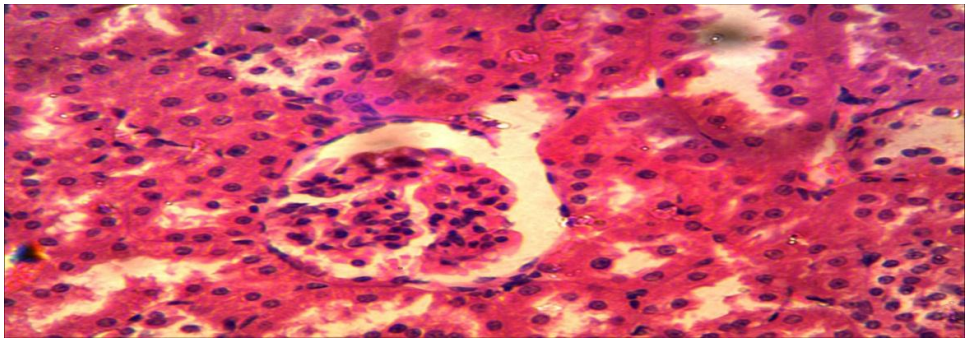

Fig. 3: Section from the rat kidney of control group showed normal appearance of glomeruli and tubules,[(H& E) X 400].

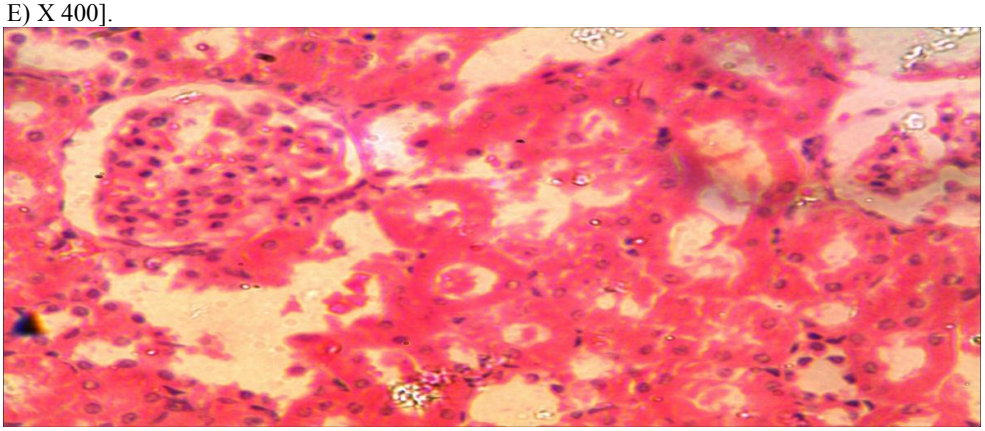

Fig. 4: Section from the rat kidney of gentamicin treated group shows dilatation of the tubules with drop out of some epithelial lining, (picture of tubular necrosis), [(H& E) X 400].

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Discussion

The present study was designed to investigate the possible potential renoprotective role of L-carnitine in gentamicin induced nephrotoxicity in rats.

Rats are suitable models of experimental animals for studying the gentamicin nephrotoxicity. This was evidenced when toxic effects of gentamicin on several enzyme activities of kidney were compared between rats and mice, it was found that rat serum urea concentration was significantly increased in gentamicin nephrotoxicity while no change occur in mice (Suzuki *et al*., 1995).

In the present work histopathologically (in gentamicin injected animals), the kidney sections showed dilatation of the tubules with drop out of some epithelial lining, (picture of tubular necrosis), (figure 4).

In accordance with Abdel-Raheem *et al*., (2010) our results in sections from control group showed normal histological structure of the glomeruli and renal tubules in the cortex and normal tubules in the medulla, (figure 3).

But in renal sections from gentamicin-treated rats, the glomeruli showed atrophy in some of them and hypertrophy in others, also there were degeneration and necrosis in the epithelial cells lining the renal tubules with cystic luminal dilatation in others at the cortex. Mononuclear leucocytes inflammatory cells infiltration was observed in focal manner between the tubules in the corticomedullary junction as well as in the perivascular area of the dilated blood vessels associated with edema, (figure 4).

These results were in line with the result obtained by Naidu *et al*., (2000) and Joel *et al*., (2002) they reported that gentamicin treated rats showed in addition to the necrosis of proximal tubules formation of hyaline casts and dilatation of distal tubules. In addition Sandhya *et al*., (1995) reported that gentamicin treated rats showed the presence of homogenous materials in the form of droplets of masses in proximal convoluted tubules in addition to inflammatory filtrates in the interstitium and these changes were markedly reduced by lipoic acid treatment.

L-carnitine is an endogenous cofactor which enhance carbohydrate metabolism and reduces the intracellular build up of toxic metabolites in ischemic conditions. L-carnitine also, is the key element for utilization of long chain fatty acids into the mitochondria to be burned into energy and in addition to its powerful antioxidant effect it is reported to have a lipid lowering effect (Martina *et al*., 1998). So, L-carnitine may provide a better renoprotective effect than the administration of the classic antioxidants.

Also in the present study L-carnitine administration to the rats injected with gentamicin showed marked improvement of renal function (significant decrease in serum urea and creatinine), (table 1 & figures 1,2).

In accordance with our results that L-carnitine administration provides renoprotective effect, Mister *et al*, (2002) revealed that L-carnitine is of value in preventing decline of renal function that occurs during ischemia reperfusion and the beneficial effect of L-carnitine possibly related to its effect of lowering lipid peroxidation and lowering of free radical generation that eventually results in the preservation of tubular cell structure.

Furthermore the renoprotective effect of L-carnitine may also be explained by its antioxidant and oxygen free radicals scavenger activity (Vanella *et al*., 2000; Dayanandan *et al*., 2001).

(Petrosa *et al*., 2005) concluded that regular L-carnitine supplementation in haemodialised patients can improve cellular defense against chronic inflammation and oxidative stress most likely by reducing some intracellular stress activated kinases such as Jun- N- Terminal kinase (JNK), an enzyme which potentially lead to peripheral blood mononuclear cell (PBMC) activation and proinflammatory cytokine production. The anti-inflammatory effects of L-carnitine are associated not with the reduction of thromboxane, and leukotriene B4, but with the increased production of prostacyclin (PGI2). Thus, the ratios between PGI2 and thromboxane, and leukotrienes B_4 tended to be higher, particularly in young animals fed L-carnitine.

On the basis of previous results, it is proved that L carnitine has renoprotective effects, so it is advised to use L carnitine in patients having renal troubles especially in ischemic nephropathy anh hypertension.

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