ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The effect of L–carnitine on gentamicin-induced nephrotoxicity and associated anemia in adult male albino rats.

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Manuscript Info

Manuscript History:

Received: 14 February 2016
Final Accepted: 19 March 2016
Published Online: April 2016

Key words:
L-carnitine, Gentamicin, Nephrotoxicity**,** hematological changes**,** antioxidant.

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Abstract

This study was conducted to investigate the possible ameliorating effect of L-carnitine against gentamicin-induced nephrotoxicity and some associated hematological changes in adult male albino rats and to exert the possible therapeutic role of L-carnitine depending on their antioxidant properities against the renal failure and its complication. A control group (n = 21) was compared with 80 mg/kg gentamicin treated rats, once daily for 10 days (n = 49). After 10 days 7 rats from each group were sacrificed for investigation. 42 nephrotoxic rats where further subdivided in to nephrotoxic rats (14 rats), nephrotoxic rats injected intraperitoneal with L-carnitine (300 mg/kg/day) for 15 and 30 days (14 rats), nephrotoxic rats injected intraperitoneal with L-carnitine (600 mg/kg/day) for 15 and 30 days (14 rats). At the end of each experiment period, 7 rats from each group were sacrificed. The effect of L-carnitine was detected by measuring urea, creatinine, β_2-microglobulin, potassium (K), osmotic fragility, total oxidant status (TOS), iron (Fe), total iron binding capacity (TIBC) and ferritin.

The effect of L-carnitine (group III and IV) was compared. The activities of biochemical parameters [urea, creatinine, β_2-microglobulin, potassium (K), total oxidant status (TOS)] and [iron (Fe), total iron binding capacity (TIBC) and ferritin] increased in nephrotoxic rats, while total protein, sodium (Na) and total antioxidant status (TAS) decreased and in haematological parameters osmotic fragility increased but haemoglobin and red blood cells (RBCs) decreased in nephrotoxic rats. Administration of L-carnitine improved alterations of biochemical and haematological parameters. In conclusion, this study demonstrated that treatment with L-carnitine attenuated the biochemical and haematological alterations induced by gentamicin and identifies new areas of research for development of better therapeutic agents for kidney and better dose. increased in nephrotoxic rats, while total protein, sodium (Na) and total antioxidant status (TAS) decreased and in haematological parameters osmotic fragility increased but haemoglobin and red blood cells (RBCs) decreased in nephrotoxic rats. Administration of L-carnitine improved alterations of biochemical and haematological parameters. In conclusion, this study demonstrated that treatment with L-carnitine attenuated the biochemical and haematological alterations induced by gentamicin and identifies new areas of research for development of better therapeutic agents for kidney and better dose.

Introduction:-

Acute renal failures, is a sudden decline in kidney function causing disturbances in fluid, electrolyte, and acid-base balance because of a loss in small solute clearance and decreased glomerular filtration rate (GFR). Even modest degrees of acute renal failure not require dialysis treatment; increase the risk of death approximately five fold **(Dennen et al., 2010).** Acute kidney injury pathogenesis is complex, and promoting events may be completely different (ischemia or toxins are major factors that precipitate in the injury), but similar pathways may be involved in subsequent injury responses **(Hur et al., 2013).**

Renal diseases are associated with a variety of haemopoietic changes. Anemia parallels the degree of renal impairment failure but the exact relationship between them remained unclear. The most important cause is failure of renal erythropoietin secretion. Other factors include depressed red cell production and reduced red cell survival **(Hales et al., 1994).**

Aminoglycosides antibiotics continue to be a main stay in clinical management of gram negative infection despite their nephrotoxicity. Gentamicin derived from gram-positive bacteria called Micromonospora purpurea present in soil and water having potential in treating aerobic gram-negative bacteria. Accumulation of gentamicin in proximal renal tubules may cause nephrotoxicity which leads to brush border network damage **(Whiting and Broun, 1996).** The nephrotoxicity involves renal free radical production and accumulation, consumption of antioxidant defense mechanisms, glomerular congestion, and acute tubular necrosis (**Abdel-Raheem et al., 2009),** leading to diminished creatinine clearance and renal dysfunction. The pathological mechanisms also involve elevation of endothelin-1, upregulation of transforming growth factor-beta (TGF-β), significant increase in monocyte/macrophage infiltration into the renal cortex and medulla, augmentation of oxidative stress, and apoptosis and also necrosis **(Ali, 1995 and Bledsoe et al., 2006).**

Oxidative stress has been recognized as an important contributory factor in a number of pathogenic processes including those affecting kidney leading to the possibility of utilizing the antioxidants for the prevention of nephrotoxicity **(Peres and Junior, 2013)**. L–carnitine is biosynthesized mainly in the liver and kidney from the amino acids lysine and methionine. The kidney plays the major role in carnitine biosynthesis, excretion, and acylation, kidney contains all enzymes needed to form carnitine from trimethyl lysine in activities exceeding that of the liver **(Cibulka et al., 2006).** L-carnitine is a compound widely distributed in nature and obtained primarily from the diet. It is involved in intermediary metabolism and is important to mammalian bioenergetic processes. It has been shown that L-carnitine plays an essential role in multiple primary functions **(Sirolli et al., 2012),** and providing a protective effect against lipid peroxidation and oxidative stress **(Ye et al., 2010).**

The major objective of this study was to investigate the possible ameliorating effect of L-carnitine against gentamicin-induced nephrotoxicity and some associated hematological changes in adult male albino rats and to exert the possible therapeutic role of L-carnitine depending on their antioxidant properities against the renal failure and its complication.

Materials and Methods:-

Drugs and Chemicals:-

Gentamicin sulfate was obtained from Memphis Co., for Pharm. and Chem. Ind. (Cairo, Egypt). L-carnitine was supplied from Mepaco-Medifood Co. (Enshas Elraml, Egypt). All other chemicals used were of good quality and analytical grade.

Animals:-

Seventy adult Male albino rats weighting 110-125 gm were selected for this study. The animals were obtained from the animal house, Theodor Bilharz Research Institute (Giza, Egypt). The animals were housed in wire cages under good ventilation condition and adequate stable diet. At least a week before use, rats were acclimated to this environment with excess food pellets and water *ad-libitum* in order to ensure that all animals were in healthy condition. Experimental protocols were approved by scientific research practice committee at Theodor Bilharz Research Institute, Giza, Egypt.

Experimental Protocol:-
The animals were divided into two main groups:

Control group (21 rats)**:** Rats in this group were injected with normal saline intraperitoneal.
Nephrotoxic rats group (49 rats)**:** Rats in this group were injected intraperitoneal with gentamicin (80 mg/ kg / day) for 10 days **(Silan et al., 2007)**

After 10 days 7 rats from each group were sacrificed by decapitation and blood samples were collected. The remaining 42 nephrotoxic rats will further subdivide in to the following subgroups:
Nephrotoxic rats + low dose of L-carnitine (14 rats)**:** Nephrotoxic rats in this group were injected intraperitoneal with low dose of L-carnitine (300 mg/kg/day) for 15 and 30 days.
Nephrotoxic rats + high dose of L-carnitine (14 rats)**:** Nephrotoxic rats in this group were injected intraperitoneal with high dose of L-carnitine (600 mg/kg/day) for 15 and 30 days.

At the end of each experiment period, 7 rats from each of the above groups were sacrificed by decapitation and blood samples were collected. One ml of blood was collected in sterile tube on potassium EDTA for hemogram by automated hematology analyzer ACT Differential (Beckman Coulter, France). The remaining blood sample were collected into tube and allowed to clot at room temperature. Thereafter, serum was separated by centrifugation at 1200_g for 15 min at 4 °C for determination of serum urea, creatinine and total protein by Hitachi 736, Hitachi, Japan. The rest of the sera were aliquoted, stored and kept frozen at -70°. Determination of sodium and potassium analysis was accomplished by emission flame photometry after suitable dilution as described by **Dean (1960).**

Determination of serum β_2-microglobulin:-
Serum β_2-microglobulin was determined using the double-sandwich ELISA technique using commercial kits MyBioSource Co., USA as described by **Cunningham et al. (1973).**
Evaluation of Erythrocyte Osmotic Fragility:
In vitro erythrocyte osmotic fragility was evaluated in all the rats in each group using the method described by **Dacie and Lewis, (1995)**, using different amounts of sodium chloride (pH 7.4) from 0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 g/L of distilled water. Briefly, freshly obtained whole blood from each rat was pipetted into the test tubes containing varying concentration of NaCl and then followed by careful, gentle mixing and incubation for 30 minutes at room temperature, 26-28◦C. The samples were then centrifuged at 600 g for 10 minutes. The supernatant was transferred into a glass cuvette and the absorbance of the supernatant measured colorimetrically with at a wave length of 540 nm. The percent haemolysis for each sample was calculated based on the formula;

$$\text{Percent haemolysis} = \frac{\text{Optical density of test solution} \times 100}{\text{Optical density of standard solution}}$$

Determination of Oxidative Stress Biomarkers (Redox data):-
1-Determination of total antioxidant status (TAS):
In this kit the reduced ABTS (2,2'–azinobis (3-ethylbenzothiazoline-6-sulfonate) molecule is oxidized to $ABTS^{\bullet+}$, using hydrogen peroxide alone in an acidic medium. In the acetate buffer solution the concentrated (dark blue-green) $ABTS^{\bullet+}$ molecules remain more stable for a long time. While it is diluted with a more concentrated acetate buffer solution at high pH, the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. The bleaching rate is inversely related to TAS of the sample **(Erel, 2004).**
2-Determination of total oxidant status (TOS):
In this kit oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ions. The oxidation reaction is enhanced by glycerol molecules, which are abundant in the reaction medium. The ferric ions make a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured by spectrophotometric, is related to the total quantity of oxidant molecules in the sample **(Erel, 2005).**

Determination of iron metabolism:-
1- *Determination of* serum iron (Fe):
Serum Fe was determined using kits from Bio-Labo. Co., France. according to the method described by **Hennesy *et al.* (1984)**.
2- *Determination of* serum ferritin:
Serum ferritin was determined using the ELISA (sandwich enzyme-linked immune sorbent assay) using commercial kits Li Star Fish, Italy, described by **Sukanya *et al.* (1981)**.
3- *Determination of* total iron binding capacity (TIBC):

Serum TIBC was determined using kits from Bio-Labo. Co., France. according to the method described by **Tietz *et al*. (1999)**.

Results:-

The serious complications resulting from gentamicin treatment are shown in table (1).

Table (1): Effects of gentamicin

Groups / Parameters	Normal control	Nephrotoxic rats	%
Urea (mg/dl)	25.840±0.770	79.030±1.340**	205.84
Creatinine (mg/dl)	0.550±0.070	2.270±0.410**	312.73
Total protein (g/dl)	7.120±0.380	5.770±0.370**	-18.96
β2-microglobulin (ng/ml)	2.350±0.220	29.780±1.790**	1167.23
Na (meq/L)	134.180±1.270	121.690±1.330**	-9.31
K (meq/L)	4.220±0.120	5.670±0.280**	34.36
Osmotic fragility initial (%)	0.520±0.101	0.750±0.070**	44.23
Osmotic fragility complete (%)	0.370±0.180	0.530±0.110**	43.24
RBCs ($x10^{12}$/L)	7.850±1.230	5.780±0.490**	-26.37
Hb concentration (g/dl)	15.001±1.620	11.350±0.520**	-24.34
TAS (mmol Trolox Equiv./L)	4.160±0.190	2.520±0.180**	-39.42
TOS (µmol H_2O_2 Equiv./L)	12.080±0.860	17.580±0.130**	45.53
Fe (µg/dL)	129.001±0.316	131.250±0.371*	1.74
Ferritin (ng/mL)	65.200±1.370	70.680±1.520*	8.40
TIBC (µg/dL)	391.801±0.374	398.001±1.414*	1.58

- Values are expressed as means ± S.E.
- %: The percentage of change of nephrotoxic rats group compared to normal control in the same row

***:**Significant difference compared to the control group at P<0.05

**: Significant difference compared to the control group at P<0.001

The effects of low and high dose of L-carnitine on nephrotoxic rats are presented in tables 2, 3 and 4.

Table (2): Effects of L-carnitine on kidney function.

Groups / Parameters	Normal control	Nephrotoxic rats	Nephrotoxic rats + low dose of L-carnitine	Nephrotoxic rats + high dose of L-carnitine
Urea (mg/dl)				
15 days	$26.001 \pm 0.440^{A}{}_{a}$	$74.600 \pm 1.330^{B}{}_{a}$	$51.700 \pm 1.210^{C}{}_{a}$	$40.300 \pm 1.220^{D}{}_{a}$
30 days	$25.001 \pm 0.580^{A}{}_{b}$	$68.540 \pm 1.520^{B}{}_{b}$	$39.700 \pm 1.190^{C}{}_{b}$	$30.400 \pm 1.330^{D}{}_{b}$
Creatinine (mg/dl)				
15 days	$0.520 \pm 0.090^{A}{}_{a}$	$1.990 \pm 0.330^{B}{}_{a}$	$1.310 \pm 0.210^{C}{}_{a}$	$0.790 \pm 0.150^{D}{}_{a}$
30 days	$0.500 \pm 0.130^{A}{}_{b}$	$1.710 \pm 0.240^{B}{}_{b}$	$0.830 \pm 0.120^{C}{}_{b}$	$0.730 \pm 0.110^{D}{}_{b}$
Total protein (g/dl)				
15 days	$6.990 \pm 0.580^{A}{}_{a}$	$5.870 \pm 0.370^{B}{}_{a}$	$6.210 \pm 0.510^{C}{}_{a}$	$6.710 \pm 0.330^{D}{}_{a}$
30 days	$7.100 \pm 0.810^{A}{}_{a}$	$6.050 \pm 0.420^{B}{}_{b}$	$6.730 \pm 0.650^{C}{}_{b}$	$6.950 \pm 0.340^{A}{}_{b}$
β_2-microglobulin (ng/ml)				
15 days	$2.340 \pm 0.480^{A}{}_{a}$	$28.720 \pm 1.130^{B}{}_{a}$	$19.350 \pm 1.130^{C}{}_{a}$	$8.900 \pm 1.330^{D}{}_{a}$
30 days	$2.350 \pm 0.720^{A}{}_{a}$	$27.500 \pm 1.320^{B}{}_{b}$	$8.500 \pm 1.270^{C}{}_{b}$	$3.100 \pm 1.150^{D}{}_{b}$
Na (meq/L)				
15 days	$135.600 \pm 1.260^{A}{}_{a}$	$124.650 \pm 1.530^{B}{}_{a}$	$126.200 \pm 1.320^{B}{}_{a}$	$130.370 \pm 1.480^{C}{}_{a}$
30 days	$136.010 \pm 1.180^{A}{}_{a}$	$126.500 \pm 1.420^{B}{}_{a}$	$131.390 \pm 1.380^{C}{}_{b}$	$134.360 \pm 1.220^{A}{}_{b}$
K (meq/L)				
15 days	$4.190 \pm 0.330^{A}{}_{a}$	$5.490 \pm 0.330^{B}{}_{a}$	$5.060 \pm 0.170^{C}{}_{a}$	$4.400 \pm 0.310^{D}{}_{a}$
30 days	$4.210 \pm 0.250^{A}{}_{a}$	$5.300 \pm 0.290^{B}{}_{b}$	$4.390 \pm 0.310^{C}{}_{b}$	$4.160 \pm 0.130^{A}{}_{b}$

-Data were represented as mean ± standard error.

-A, B, C, D: means bearing different superscripts within the same row are differ significantly ($P<0.05$).

-a, b: means bearing different superscripts within the same column are differ significantly ($P<0.05$).

Table (3): Effects of L-carnitine on redox data, RBCs, Hb and osmotic fragility

Groups / Parameters	Normal control	Nephrotoxic rats	Nephrotoxic rats + low dose of L-carnitine	Nephrotoxic rats + high dose of L-carnitine
TAS (mmolTrolox Equiv./L)				
15 days	$4.150 \pm 0.360^{A}_{a}$	$2.720\pm0.210^{B}_{a}$	$3.490\pm0.390^{C}_{a}$	$3.840\pm0.190^{D}_{a}$
30 days	$4.170 \pm 0.180^{A}_{a}$	$2.880\pm0.340^{B}_{b}$	$3.890\pm0.360^{C}_{b}$	$4.040\pm0.440^{D}_{b}$
TOS (μmol H_2O_2 Equiv./L)				
15 days	$11.900 \pm 0.330^{A}_{a}$	$17.200\pm0.630^{B}_{a}$	$14.001\pm0.630^{C}_{a}$	$12.880\pm0.660^{D}_{a}$
30 days	$11.830 \pm 0.580^{A}_{a}$	$17.020\pm0.110^{B}_{a}$	$12.560\pm0.860^{C}_{b}$	$11.860\pm0.730^{A}_{b}$
RBCs ($X10^{12}$/L)				
15 days	$7.770 \pm 0.880^{A}_{a}$	$5.900\pm0.160^{B}_{a}$	$6.790\pm0.330^{C}_{a}$	$7.050\pm0.510^{D}_{a}$
30 days	$7.900 \pm 0.580^{A}_{a}$	$6.100\pm0.410^{B}_{b}$	$7.150\pm0.420^{C}_{b}$	$7.570\pm0.830^{D}_{b}$
Hb concentration (g/dl)				
15 days	$14.870 \pm 1.330^{A}_{a}$	$11.440\pm0.480^{B}_{a}$	$12.860\pm0.190^{C}_{a}$	$14.180\pm0.510^{D}_{a}$
30 days	$15.030 \pm 1.880^{A}_{a}$	$11.730\pm0.430^{B}_{a}$	$14.090\pm0.610^{C}_{b}$	$14.640\pm0.650^{A}_{b}$
Osmotic fragility initial (%)				
15 days	$0.530 \pm0.020^{A}_{a}$	$0.740\pm0.040^{B}_{a}$	$0.640\pm0.010^{C}_{a}$	$0.540\pm0.010^{D}_{a}$
30 days	$0.520\pm0.020^{A}_{a}$	$0.710\pm0.030^{B}_{b}$	$0.540\pm0.010^{C}_{b}$	$0.500\pm0.030^{A}_{b}$
Osmotic fragility complete (%)				
15 days	$0.370 \pm 0.020^{A}_{a}$	$0.510\pm0.110^{B}_{a}$	$0.440\pm0.020^{C}_{a}$	$0.380\pm0.010^{D}_{a}$
30 days	$0.380\pm 0.020^{A}_{a}$	$0.500\pm0.050^{B}_{a}$	$0.390\pm0.010^{C}_{b}$	$0.360\pm0.010^{A}_{b}$

-Data were represented as mean ± standard error.
-A, B, C, D: means bearing different superscripts within the same row are differ significantly (P<0.05).
-a, b: means bearing different superscripts within the same column are differ significantly (P<0.05).

Table (4): Effects of L-carnitine on iron metabolism

Groups / Parameters	Normal control	Nephrotoxic rats	Nephrotoxic rats + low dose of L-carnitine	Nephrotoxic rats + high dose of L-carnitine
Ferritin (ng/mL)				
15 days	$66.001 \pm 0.580^{A}_{a}$	$70.800\pm0.580^{B}_{a}$	$68.630\pm0.190^{C}_{a}$	$68.370\pm0.190^{C}_{a}$
30 days	$65.500\pm 0.810^{A}_{a}$	$69.500\pm0.630^{B}_{a}$	$67.120\pm0.390^{C}_{a}$	$65.830\pm0.390^{A}_{b}$
Fe (μg/dL)				
15 days	$128.666 \pm 0.166^{A}_{a}$	$130.633\pm0.185^{B}_{a}$	$129.710\pm0.033^{C}_{a}$	$128.270\pm0.194^{A}_{a}$
30 days	$128.500\pm 0.577^{A}_{a}$	$129.800\pm0.707^{B}_{a}$	$128.698\pm0.265^{C}_{b}$	$128.377\pm0.292^{A}_{a}$
TIBC (μg/dL)				
15 days	$391.333 \pm 0.333^{A}_{a}$	$396.733\pm0.968^{B}_{a}$	$395.450\pm0.165^{C}_{a}$	$394.266\pm0.194^{C}_{a}$
30 days	$392.000 \pm 0.577^{A}_{a}$	$395.600\pm0.815^{B}_{a}$	$393.500\pm0.500^{C}_{b}$	$392.888\pm0.351^{A}_{b}$

Data were represented as mean ± standard error.
-A, B, C, D: means bearing different superscripts within the same row are differ significantly (P<0.05).
-a,b: means bearing different superscripts within the same column are differ significantly (P<0.05).

eewteb noitalerroc ehTn L-carnitine and different studied parameters are presented in table 5

Table (5): correlation between L-carnitine and different studied parameters

Correlations	low dose of L-carnitine Correlation coefficients	high dose of L-carnitine Correlation coefficients
L-carnitine vs urea	**-0.96****	**-0.98****
L-carnitine *vs* creatinine	**-0.99****	**-0.91****
L-carnitine *vs* total protein	**0.96****	**0.96****
L-carnitine *vs* β_2-microglobulin	**-0.99****	**-0.95****
L-carnitine *vs* Na	**0.96****	**0.99****
L-carnitine *vs* K	**-0.99****	**-0.95****
L-carnitine *vs* osmotic fragility initial	**-1.00****	**-0.91****
L-carnitine *vs* osmotic fragility complete	**-0.99****	**-0.91****
L-carnitine *vs* RBCs	**0.96****	**0.97****
L-carnitine *vs* Hb concentration	**0.99****	**0.94****
L-carnitine *vs* TAS	**0.98****	**0.92****
L-carnitine *vs* TOS	**-0.96****	**-0.92****
L-carnitine *vs* Fe	**-0.98****	**-0.95****
L-carnitine *vs* Ferritin	**-0.91****	**-0.99****
L-carnitine *vs* TIBC	**-0.99****	**-0.94****

** Correlation is significant at the 0.001 level

Discussion:-

Gentamicin-induced nephropathy is a limiting factor for its clinical usage. Results from many studies have shown that gentamicin produced an elevation in the concentrations of biochemical indicators of kidney function such as serum urea, creatinine and total protein excretion in urine and elevation in β2-microglobulin concentration **(Pedraza-Chaverri et al., 2004; Gonick, 2008).** Consistent with the data from these studies we observed in our study that urinary excretion of total protein was increased after gentamicin injection indicating tubular damage. On the other hand, urea and serum creatinine levels were augmented indicating glomerular damage. Our data also revealed significant increase in serum level of β2-microglobulin concentrations. **(Adeyemi at al., 2001; Gonick, 2008)** reported that**,** appearance of β_2-microglobulin in the urine depends on plasma β_2-microglobulin elevation exceeding its renal reabsorptive threshold of 5mg/l and/or from proximal tubular damage.

Serum electrolytes were disturbed significantly in gentamicin treated rats as compared with normal control rats. Gentamicin may cause Na^+ loss due to hyper dynamic circulation which in turn leads to the observed increased in serum K^+ level in this study. These results are in agreement with **Lobetti and Joubert, (2000) and Saleh (2014).** Moreover, these results are in harmony with **Heibashy et al., (2010).** The last auther demonstrated that, lower value of Na^+ indicates inability of kidney to conserve Na^+ and chloride. Haemodillution may be involved in the fall of Na^+value *via* excess of water intake and or increased production of endogenous water. In turn, the reversed increases of K^+ appeared may be due to reduced excretion of K^+ aggravated by leakage of intracellular K^+ in to blood stream as a results of gentamicin induced lesions in renal tubular epithelium.

The toxicity of gentamicin is believed to be related to the generation of reactive oxygen species (ROS) in the kidney **(Martinez-Salgado et al., 2004)**. The results of present study revealed significant increase in TOS and decrease in TAS in nephrotoxic group compared to normal control group. These results are in harmony with studies of **Hozayen et al. (2011)** who found that gentamicin administration to rats enhances the production of hydrogen peroxide in renal cortical mitochondria as a result of the increase in the production of superoxide anions. Superoxide anion and hydrogen peroxide may interact to form a reactive and unstable radical, namely a hydroxyl radical **(Lobo et al., 2010)**. This radical is formed by the reaction between hydrogen peroxide and Fe2+ (**Kocha et al., 1997)**. $Fe2^+$ appeared to play an important role in the production of reactive oxygen radicals in gentamicin nephrotoxicity and when oxygen radicals begin to accumulate; renal cells exhibit a defensive mechanism by using various antioxidant enzymes; such as (Catalase) CAT, (Superoxide dismutase) SOD and (Glutathione peroxidase) GPx activities (**Wang et al., 2005)**. Reduced activity of one or more antioxidant systems, due to the direct toxic effect of gentamicin or volume depletion due to gentamicin administration, leads to an increase in lipid peroxidation. The decreased amount of intracellular GSH and the accumulation of hydrogen peroxide and hydroxyl radicals are the triggering factors in gentamicin nephrotoxicity **(Erdem et al., 2000)**. Moreover, **Yaman and Balikci et al. (2010)** reported a highly

significant decrease in SOD and CAT activity after gentamicin administration. **Parlakpinar et al. (2005)** showed that gentamicin suppresses antioxidant defense enzymes and increases lipid peroxidation in the kidney. This gives rise to membrane lipid damage and initiation of autocatalytic reactions. The damage in plasma membrane results in loss of osmotic balance and intracellular calcium levels increase. Gentamicin administration to normal rats caused severe damage to renal tissues most likely by ROS mediated mechanism as evident by decreased activities of above antioxidant enzymes and total SH levels that led to increased lipid peroxidation (**Yazar *et al*., 2003; Banday *et al*. 2008**).

Hematological constituents usually reflect the physiological responsiveness of the animal to its external and internal environments and this is serving as a veritable tool for monitoring animal health. Hematological profile in animals is an important indicator of physiological or pathophysiological status of the body **(Elsayed, *et al*., 2014)**. Our data showed that Anemia has been observed following administration of gentamicin in albino rats. Gentamicin induced a highly significant decrease in Hb concentration and RBCs. These results are in line with data obtained by **Debska-Slizien *et al*., (2011) and El-Maddawy (2014)**. Several mechanisms could contribute to the anemia observed after gentamicin induced renal failure. Erythropoietin (Epo) is the major physiological regulator of erythropoiesis. The primary site of Epo is thought to be the renal peritubular cells. It has been reported that regulation to Epo production is related to proximal tubular function. Therefore, one of the major causes of the Anemia induced by gentamicin treatment is Epo deficiency following injury to the kidney **(Naeshiro *et al*., 1997)**. **Nagano *et al*. (1990)** reported that a gentamicin-treated rat is a useful and convenient anaemic model and recombinant human erythropoietin (r-HuEPO) is useful for treatment of anemia in acute renal failure. Anemia may also results from hemodilution, extra vascular hemolysis as well as from toxic dyshematopoiesis **(Elyazji and Abdel-Aziz, 2013)**. Anemia may be the result of lipoperoxidative changes in erythrocyte membrane **(Ambali *et al*., 2010)**.

Erythrocytes have been used extensively as a model system for investigating mechanisms of oxidative stress since these cells, lacking protein synthesis machinery and represent a simplified model **(Battistelli *et al*., 2005)**. In addition, The RBC is vulnerable to lipoperoxidative changes because of its direct association with molecular oxygen, high content of metal ions catalyzing oxidative reaction and availability of high amount of polyunsaturated fatty acids (PUFA), which are susceptible to lipid peroxidation (**Wadhwa *et al*., 2012)**.

Oxidative stress which results from the generation of free radicals and alteration in free radical scavenging system is one of the complex factors that determine the integrity of the erythrocyte **(Uzum *et al*., 2006)**. **Chidiebere *et al*. (2011)** showed that the destruction of RBCs is postulated to occur by either membrane oxidation or Hb denaturation. The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of RBC. In our study the increase in the erythrocyte osmotic fragility in nephrotoxic rats confirmed the observation that this can be used as indirect method of evaluating lipid peroxidation in animals. Process of lipid peroxidation decreases hydrophobic characteristic of bilayer membrane of erythrocyte altering affinity and interaction of protein and lipids, thereby impairing the functioning and homoestasis of erythrocyte membranes **(Ambali *et al*., 2010)**. Plasma levels of lipid peroxidation products correlate with the severity of renal Anemia **(Horl, 2002)**. Generation of oxygen free radicals may result in cross-linking within membrane proteins and/ or between membrane proteins and Hb. Protein degradation was found to be increased in RBC exposed to oxygen free radicals, with RBC morphological changes accompanying these structural alterations; echinocyte formation is a characteristic morphological change due to oxidant damage **(Kusmic *et al*., 2000)**. Mechanical stability of RBC membranes may also be influenced by oxidant attack, and instability may result from oxidative alterations in certain membrane skeletal proteins **(Lin and Beiyi, 2008)**. It has been previously reported that oxidants can alter surface characteristics of RBC **(Shi and Pamer, 2011)**, by a decreased cytoskeletal protein content and production of high-molecular-weight proteins, which can lead to abnormalities in erythrocyte shape and rheologic properties **(Battistelli *et al*., 2005)**. Additionally, oxidative damage may affect the transport processes through the RBC membrane, affecting the cell geometry and cytosolic viscosity, accompanied by the alterations in the cytosolic concentration of cations. Calcium homeostasis is an important determinant of normal RBC deformability; increased cytosolic calcium concentration leads to impaired RBC deformability **(Toptas *et al*., 2006)**.

The data obtained from present study showed that gentamicin induced a significant increase in serum iron, ferritin and TIBC. In vivo, most of the iron is bound to heme and non heme proteins and does not directly catalyze the generation of the hydroxyl radical **(Baliga *et al*., 1998)**. The in vitro and in vivo studies indicate enhanced generation of hydrogen peroxide and release of iron in response to gentamicin. Based on the ability of superoxide to release iron from the iron-storage protein ferritin (which normally provides a secure mean for iron storage in the

inert form), ferritin has been suggested as a possible source of iron for the generation of powerful oxidant species. Ferritin is an acute-phase reactant, its synthesis is up-regulated by infection or inflammation **(Pourabbas *et al*., 2013 and El-Khatib, 2009)**. Similar increase in serum ferritin was detected in patients with acute renal failure **(Gulcelik and Kayatas, 2002 and Branten *et al*., 2004)**. Moreover, Gentamicin was shown to chelate mitochondrial iron mediated by hydrogen peroxide forming iron-gentamicin complex which is a potent catalyst of free radical formation capable of inhibiting mitochondrial respiration causing cell death **(El-Maddawy, 2014)**. In addition, Fe levels increased in serum as a result of increased hemolysis of RBCs this in line with **Crook, (2012)** who demonstrated that in hemolysis condition ferritin and Fe levels increased. TIBC increased to bind Fe which liberated from RBCs destructed. Iron has a major role in the initiation and propagation of lipid peroxidation, either by catalyzing the conversion of primary oxygen radicals to hydroxyl radicals or forming a perferryl iron. In addition, iron can directly catalyze lipid peroxidation, the oxidative reaction of polyunsaturated lipids, by removing hydrogen atoms from PUFA in the lipid bilayers of organelle membranes **(Shah *et al*., 2007).** Multiple independent pathways exist which converge in the increase of ferritin synthesis in response to various forms of oxidative insult. Ferritin, with its ability to oxidize and sequester intracellular iron in an internal mineral core, limits the levels of catalytically available iron, owing to the generation of free radicals, as a critical cytoprotective protein that constitutes an integral part of the antioxidant response (**Traina, 2011)**.

The knowledge of the mechanisms responsible for aminoglycosides-induced renal damage has prompted the development of potential strategies to prevent its nephrotoxicity (**Reisfeld *et al*., 2011).** The most used strategies to prevent aminoglycosides toxicity have been: a) blocking aminoglycosides binding to membranes and aminoglycosides uptake; b) preventing phospholipidosis, c) reducing oxidative stress, d) antagonizing vasocontrictor effects; d) preventing inflammation **(Servais *et al*., 2008)**. Recent studies have shown that natural antioxidants obtained from different alternative systems of medicine display a wide range of biological activities. Various alternatives possessing antioxidant properties have been used in order to minimize gentamicin induced oxidative stress in animal models **(Khan *et al*., 2011)**.

In the present study L-carnitine was administered in low dose (300 mg/kg/day) and high dose (600 mg/kg/day) in order to provide some assurance that if no ameliorating effect of L-carnitine was identified, the lack of effect could not be ascribed to an inadequately small dose. Data revealed significant improvement in both urea and creatinine level which was dependently related to L-carnitine doses. Our data was in agreement with **Ustundag *et al*. (2009) and Gebaly *et al*. (2012)** who reported that this improvement seem to presumably be due to the amelioration of gentamicin induced oxidative injury to the tubular system and significant increase in total protein. The mechanism by which this antioxidant performed it is action may be due to it is membrane stabilizing effect and ROS scavengers. This is in agreement with the results of **Duranay *et al*. (2006).** The last authors reported that the increase of total protein might be caused by improved anabolic effects or anti-inflammatory activities of L-carnitine. In kidneys, L-carnitine decreased the severity of renal cortical proximal tubular necrosis and improved renal function in rats with gentamicin-induced or doxorubicin-induced renal injury **(Boonsanit *et al*., 2006).** β2-microglobulin were corrected significantly in current study. The improvement in serum β2-microglobulin dependently related to L-carnitine doses. This is in agreement with the results of **Ustundag *et al*. (2009).** The last authors reported that improvement seem to presumably be due to the amelioration of gentamicin induced oxidative injury to the tubular system. In current study, L-carnitine low and high dose induced a great improvement in serum Na^+ and K^+ levels. The improvement in serum Na^+ and K^+ levels dependently related to L-carnitine dose, this improvement might be due to L-carnitine had protective effect on lipid peroxidation by reducing formation of hydrogen peroxides and MDA, and it improved antioxidant status in rats. Moreover, it increased free radical scavenging from the cellular sites **(Sener *et al*., 2004).**

Data of the present study revealed significant increase in TAS and decreases in TOS in rats treated with both low and high dose of L-carnitine. L-carnitine inhibits superoxide radicals, and reduces lipid peroxidation catalyzed by hydrogen peroxide. It is also proposed that L-carnitine can reduce the production of hydroxyl radicals generated by iron, because of its iron-chelating activity **(Vanella *et al*., 2000). Boonsanit *et al*. (2006)** also reported improvement of kidney functions and the correction of decreased level of oxidative stress enzymes in the form of SOD, CAT and Serum GSH by the use of L- carnitine. **Mansour (2013)** also showed that L-carnitine improved endothelial responses by decreasing ROS production and increasing nitric oxide availability. L-carnitine also could help the cells to repair single-strand breaks induced in DNA and to protect it from oxygen free radicals **(Alzahrani, 2011)**.

L-carnitine maintains membrane stability; this has been shown most clearly in erythrocytes. Rats treated with L-carnitine in the present study showed significantly increase in Hb concentration and RBCs. Effect of L-carnitine on

Hb content was parallel to its action on RBCs count. Moreover, data in present study revealed that treatment with L-carnitine ameliorated the increase in erythrocyte fragility following gentamicin treatment. Our results were in accordance with **Horl (2002); Malaguarnera *et al.* (2011) and El-Maddawy (2014)** who reported that treatment with L-carnitine improved hematological parameters due to its antioxidant properties that enhanced hematopoiesis. **Nikolaos *et al*. (2000) and Nand *et al*. (2008)** also noticed similar results in hemodialysis patients treated with L-carnitine supplementation. L-carnitine plays an important role in the cellular metabolism by participating to the structural units of erythrocyte membrane (lipids and proteins such as spectrin and actin) and to the mitochondrial oxidative degradation of long chain fatty acids **(Karadeniz *et al*., 2008)**. L-carnitine was found to protects erythrocytes from oxidative stress, prevents lipid peroxidation and stabilizes the cell membrane, raises RBC osmotic resistance, reduces deformability of RBC, increasing the life span of RBCs and was found to inhibit apoptosis in different diseases **(Ragab and Mahfouz, 2010 and Kudoh *et al.*, 2014).** Moreover, **Chidiebere *et al.* (2011)** reported that L-carnitine increases the action of EPO on bone marrow. The activity of Na^+−K^+-ATPase in erythrocytes is inhibited by the addition of uraemic plasma **(Ambali *et al*., 2010)**, which reduces the number of pump sites, particularly in young erythrocytes. L-carnitine has a key role in Na^+−K^+ pump activity, instrumental in the maintenance of the biconcave discoid shape of erythrocytes. Circulating free fatty acids are endogenous inhibitors of the Na^+−K^+-ATPase. L-carnitine increases delivery of free fatty acid to the mitochondria for oxidation and as a result the plasma concentration of free fatty acids decreases and the inhibition of the Na^+−K^+-ATPase was reversed **(Uchendu *et al*., 2011).** Indeed the direct L-carnitine addition to whole blood in vitro delayed haemolysis **(Karadeniz *et al*., 2008).**

The noticed ameliorating effect of L-carnitine on RBCs count, RBCs membrane stability and free radical scavengers may explain the significant decrease in serum ferritin, serum Fe and TIBC levels detected in the present study. This is in agreement with **Matsumoto *et al.* (2001)** who reported that significant decrease in Fe may related to that L-carnitine had beneficial effects on stabilized cellular membranes prolongs their lives and raises RBC osmotic resistance. Moreover, **Traina (2011)** reported that L-carnitine down regulates ferritin-H gene expression. L-carnitine suppressed hydroxyl radical production probably by chelating the iron required for the generation of hydroxyl radicals. Furthermore, the preventive effect of L-carnitine on the formation of ROS due to the xanthine /Xo system has been demonstrated (**Mohamed and Farghaly, 2009)**. L-carnitine exerts antioxidant effect and reverses iron-induced oxidative stress in human fibroblast. It is possible that L-carnitine might reduce available iron, by reducing ferritin expression (**Traina, 2011).**

The present study revealed the nephrotoxic effects of gentamicin and its hazardous influence on RBCs count and RBCs membrane stability. The use of L-carnitine in combination with gentamicin minimized its toxicity as revealed from decreasing urinary excretion of total protein, serum urea, creatinine and β2-microglobulin levels. In addition, L-carnitine had been proved to have an ameliorating effect the on gentamicin-induced Anemia and enhanced erythrocyte membrane stability and RBCs osmotic resistance. Oxidative stress reactions, ROS and release of iron may be one of the mechanisms of gentamicin-induced nephrotoxicity. The ameliorative effect of L-carnitine against gentamicin-induced toxicity may be at least in part due to its antioxidant and free radicals scavenger properties and may indicate the utility of adjuvant L-carnitine treatment for renal toxicity and anemia management in gentamicin treated patients.

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