The Protective Effect of L - Carnitine on Paracetamol-induced Nephrotoxicity in Adult Male Albino Rats (Microscopic and Biochemical studies)

Zeinab Mahmoud Gebaly[1], Basma Kamal Ramadan[2] and Mona Hussein Hamouda[1]

[1]Histology and [2]Physiology Departments, Faculty of Medicine for girls, Al-Azhar University
zienabgebaly@live.com

Abstract: Background: L-Carnitine, antioxidant agent, have protective effects againt lipid peroxidation.It is a cofator in the transfer of long-chain fatty acid allowing the beta-oxidation of fatty acid in the mitochondria. **Aim of the work:** This study aims to investigate the antioxidant effect of L- Carnitine, on acute nephrotoxicity induced by paracetamol overdose and to understand the mechanism of prevention of this toxicity. **Material and methods:** Four groups of rats (n= 7 in each group) were used. The animals in the control group (group I) did not receive any treatment. The animals in group II received 500 mg/kg b.w./day of L- Carnitine 7 days orally using gastric gavage tube. The animals in group III orally received paracetamol powder dissolved in 50% propylene glycerol as a single dose of 640 mg/kg b. w. Animals in the last group (group IV) were pretreated with oral L- Carnitine for 7 days at a dosage of 500 mg/kg before paracetamol administration. Both kidneys in all rats were removed and tissue SOD and CAT were evaluated. Serum GSH, urea and creatinine, in addition to the histological evaluation using hematoxylin – eosin staining and electron microscope studies were also determined. **Results:** Renal SOD and CAT, serum urea and creatinein were higher in group III compared to group I and II ($p < 0.05$), while serum GSH showed a significant reduction in this group. Pretreatment with L-Carnitine prevent these changes. Histologically, paracetamol caused massive degenerative changes in the kidney tissue which was confirmed by quantitative microscopy and the electron microscopic findings. These changes were attenuated by L – Carnitine pretreatment. **Conclusion:** Paracetamol overdose resulted in signs of kidney damage which was evident by an increase in the tissue level of SOD, serum urea and creatinin with a significant reduction in GSH level. Pretreatment with L- Carnitine seems to attenuate the renal damage, as evidenced indirectly by low SOD level.

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Key Words: SOD: superoxide dismutase **CAT:** Catalase **GSH:** glutathione**.**

1. Introduction

Drug induced nephrotoxicity is a serious and common danger. The use of nephrotoxic drugs has been implicated as a causative factor in up to 25% of all cases of severe acute renal failure in critically ill patients **(Pannu and Nadim, 2008)**. This is probably because the kidney is supplied with a large volume of blood accounting for 20% of total cardiac output. Therefore, the kidney is likely to be affected by secondary effects of drugs and their metabolites that are concentrated through the urine concentrating mechanism **(Marieb, 2006).**

Acetaminophen (paracetamol) is widely used as a safe antipyretic and analgesic drug ***(*Ozkaya *et al.,* 2010)**. However, paracetamol (APAP) is now considered as the most common drug in self-poisoning, with a high rate of morbidity and mortality (**Sheen *et al*., 2002**)[i]. It has been reported that an overdose of acetaminophen has life-threatening effects on liver and kidney ***(*Whitcomb and Block, 1994*; Ghosh and Sil, 2007; Demirbag and Uysal,* 2010)** Availability of paracetamol as an over counter (OTC) medication alone and in combination with other prescription creates a situation that may lead to exposure to excessive quantities of the drug ***(*Shayiq *et al.,* 1999*).*** In sensitive individuals, such as persons with renal insufficiency, therapeutic doses of paracetamol have also been implicated in kidney damage ***(*Stern *et al.,* 2005*).***

The nephrotoxicity induced by paracetamol overdose was characterized by damage and necrosis in the proximal tubule ***(*Abdel – Zaher *et al.,* 2008**), degrees of tubular obstruction and vacuolization of tubular cells with cellular debris ***(*Isik *et al.,* 2006*)*** in addition to loss of proximal tubular brush borders, mitochondrial damage and basement membrane disruption ***(*Satirapoj *et al.*, 2009*).***

Paracetamol (APAP) is normally metabolized in the liver to glucoroide, sulfate conjugates, small amounts of hydroxylated and deacetylated metabolites. Small amounts, about 10%, are metabolized by cytochrome p-450 especially in the cells of the renal proximal tubules. This pathway involves the formation of a highly reactive intermediate namely N.acetyle-p-benzoquinoneimine (NAPQI) ***(*Benson *et al.,* 2005*)***. This intermediate normally conjugates with reduced glutathione (GSH)

and / or protein sulphydryl groups to render it intermediate harmless **(James *et al.*, 2003)**. The conjugate is subsequently transformed to cysteine and mercapturic acid conjugates and thus removed from hepatocytes **(Slitt *et al.*, 2005)**.Overdose with APAP depletes cellular GSH, resulting in the binding of NAPQI to mitochondrial proteins. This is followed by mitochondrial dysfunction, ATP depletion, oxidative stress, DNA damage and oncotic necrosis of parenchymal cells **(Kaplowitz, 2004; Jaeschke *et al.*, 2011)**. These acetaminophen (APAP) metabolites can reach the kidney through bile excretion which is completely reabsorbed from the intestine and thereby reach the kidney and contribute to the total excretion of paracetamol and its metabolites in the urine **(Blantz, 1996)**.

Several studies indicated that oxidative stress plays a major role in nephrotoxicity induced by paracetamol overdose. It is of interest, therefore, to evaluate the role of antioxidants in attenuation of paracetamol nephrotoxicity (no refrence).

L-Carnitine(B-hydroxy-(y-N-trimethylamino) butyrate, carnitine), which is an amino acid derivative that facilitates the transfer of long-chain (>10carbon) fatty acids into the mitochondria of skeletal muscle and cardiomyocytes, where they undergo beta-oxidation. By this mechanism carnitine profoundly influences both skeletal muscle and myocardial fatty acid oxidation, and maintains low pools of fatty acid (acyl)-coenzyme A compounds, which are potentially toxic **(Bremer,1983 and Joel *et al.*, 2002)**. It is synthesized endogenously from the essential amino acid, methionine and lysine in the liver and kidneys and stored in the skeletal muscle, heart, brain and sperm **(Lheureux and Hantson, 2009)**. This antioxidant can prevent the accumulation of end products of lipid peroxidation, thus it may protect against kidney damage and has been proposed as a treatment for many conditions because it reduces oxidative stress **(Aydogdu *et al.*, 2006)**.

Therefore, the aim of the present study is to shed more light on the possible nephro protective effect of L- Carnitine against acute nephrotoxicity induced by paracetamol overdose. The effect of the antioxidant L- Carnitine in paracetamol induced toxicity was evaluated by determining the levels of creatinine and blood urea. The activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and Catalase (CAT) were measured in kidney homogenates. Further, the level of serum glutathione (GSH) was also estimated. To assess the microscopic structural changes of the kidney, histological examination studies were conducted on kidney sections of normal, L- Carnitine treated, paracetamol treated rats, and rats treated with L- Carnitine before and after paracetamol adminstration.

2. Materials and Methods

(I) Animals:

Twenty eight adult male Sprague Dawley albino rats (120-180 gram body weight) were used. They were housed in stainless steel cages at room temperature, regular light cycle 12: 12 h. light: dark cycle and fed rodent chow and water . They were fasted 17 hours (4: 00 p.m- 9: 00 A.m) before the experiments, but they were allowed free access of water.

(II) Experimental procedures and treatment:

Rats were kept singly in plastic cages during the experimental period. One week acclimatization period was allowed before initiation of the experiment. On the start of the 2nd week, experimental groups were divided into four equal groups:

Group I: (Control group): 7 rats were supplied with access water and ordinary rat chow.

Group II: (L-Carnitine group): 7 rats received 500 mg/kg L-

Carnitine (**Yaper *et al.*, 2007**) by oral route using a gastricgavage tube, for 7 days.

Group III: (Acute paracetamol(Acute APAP) group): 7 rats received a single oral dose (640 mg/kg) paracetamol dissolved in 50% propylene glycol /using gastric gavage tube (**Devi *et al.*, 2005**). The animals were treated between 9:00 and 10.00 A.M to minimize the possible diurnal effects in tissue glutathione concentration influencing the experimental results.

Group IV: (Acute paracetamol group pretreated with L- Carnitine (L-Carnitine+ Acute APAP) group)7 rats were administerd L-Carnitine 500 mg/kg/day for 7 days by gastric gavage tube. On the 8th day, a single oral dose of paracetamol 640 mg/kg b.w.was administrated by gastric gavage tube.

Blood and tissue samples were collected 72 hours after paracetamol administration, in groups of rats treated with acute paracetamol (III) and with acute paracetamol after L- Carnitine treatment (IV).

(III) Hematological study:

At the end of the experiment, animals were fasted for 12-14 hours before collection of blood samples. Blood was collected from retro – orbital sinus by heparinized capillary tubes under light ether anesthesia **(Simmons and Brick, 1970)**. The blood was collected in a centrifuge tubes and allowed to clot for an hour at room temperature and then centrifuged at 3000 rpm for 15 minutes. The sera were, then separated and stored at – 20 C^{o} (using - 20 Co REVCO refrigerator).

Serum Assay:

The separated sera were analyzed for estimation of:

- Serum glutathione (GSH) using Cayman's GSH assay kit,

- Serum urea using modified Berthelot – searcy method *(***Henry, 1991***)* .
- Serum creatinine activity using the application of Jafe reaction *(***Wilson and Walker, 2000***).*

(IV) Tissue Sampling

After blood sampling, animals were anaesthized by ether. A dorsal midline incision was done with dissection of the muscle to reach renal bed and expose the kidneys.

Careful dissection was done to the renal pedicle and it was cut rapidly. The right kidney was used for histological studies and the left was used for tissue enzymology.

(a) Preparation of renal homogenate:

The left kidney was dissected free from the fat and connective tissue. Each kidney was longitudinally sectioned, and the renal cortex was dissected out and homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4 C^o. The resulting supernatant (cytosolic fraction) was used for the determination of two antioxidant enzymes. Determination of catalase (CAT) activity using *(*Clair – Borne, 1985*)* method; and super oxide Dismutase (SOD) activity using *(*Mark – Lund, 1985*)* method. All tests were done according to the instructions provided.The activities were estimated using colorimetric assay.

(b) Histological examination:

1. For light microscopy: the specimens were fixed in 10% neutral buffered formol then processed to obtain 6 um – thick paraffin sections. The sections were stained with Harris, haematoxylin and eosin stain (Durry and Wallington, 1980*)* for routine histological examination.
2. For electron microscopy: the specimens were immediately fixed by immersion in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer, at 0-4 C^o and pH 7.3 *(*Merser and Birbeck, 1966). The specimens were then washed for 1.5 hour with 3 changes of the same buffer. Then fixed in 1% Osmiun tetraoxide in the same cacodylate buffer for 2 hours (Merser and Birbeck, 1966*).*

Quantitative Histology

Ten histological sections representing each of the control and treated groups were subjected to quantitative measurement of the percent vacuolated areas using Image Pro plus image analysis software.

Statistical Analysis:

Results were expressed as mean standard error and statistically analyzed using the student's "t" test according to Kirwood (1989*).*

3. Results

As shown in tables (1 and 2) there were no significant changes in serum urea, creatinine and GSH between normal and L-Carnitine treated groups. Also, renal CAT and SOD showed insignificant changes between the two groups.

Effect of L- Carnitine supplementation on serum urea, creatinine and GSH concentrations:

Serum urea and creatinine concentrations were significantly increased ($P<0.05$) in the acute paracetamol treated group(acute APAP) of animals compared to the normal control group. Treatment with L- Carnitine prior to paracetamol administration (L-Carnitine+APAP group) showed significant ($p<0.05$) decrease in their concentrations compared to the paracetamol treated group. At 72 hours following paracetamol treatment, the serum level of GSH was significantly reduced ($P<0.05$) to nearly 40% of the control value. Treatment with L-Carnitine prevented GSH reduction. There was a significant ($p<0.05$) increase (about 62%) when compared to serum GSH content of paracetamol treated group.

Effect of L – Carnitine supplementation on kidney antioxidant status:

Renal SOD activity, was significantly ($p <0.05$) lower in 72 hours paracetamol treated group than that of control (Table 2) . Pretreatment with L-Carnitine 7 days before paracetamol administration significantly ($P< 0.05$) prevented the decreased in SOD activity compared to the paracetamol treated rats. Likewise, the decrease in the CAT activity as a result of the treatment with paracetamol was also restored by the L- Carnitine ($p<0.05$) pretreatment when compared to the paracetamol treated animals.

3) Histological Examination

Light Microscopic Results:

Control Group:

Light microscopic examination of control kidney rats sections stained with haeamatoxylin and eosin revealed the presence of normal renal corpuscle and tubules in the renal cortex. Each renal corpuscle appeared as oval or rounded structures each one was formed of a glomerulus and Bowman's capsule. The glomerulus contained tuft of capillaries lined by flat endothelial cells and surrounded by a double walled Bowman's capsule with a filtration space in between. The renal tubules were near to each other and surrounded the renal corpuscles.The proximal convoluted tubule (PCT) was lined by simple cubical epithelium. The distal convoluted tubule(DCT) was lined by low simple cuboided epithelium (Fig.1).

L-Carnitine Group:

Light microscopic examination of the kidney specimens of L-Carnitine treated animals, showed no detectable pathological changes. The glomeruli and the tubules showed normal appearance (Fig.2).

Table 1. Effects of acute paracetamol and L-Carnitine treatments on serum urea, creatinine and glutathione (GSH) levels.

Groups / Parameters	Normal Control group		L-Carnitine treated group compared to Normal control group			Acute paracetarmol (Acute APAP) group compared to normal control group			L-Carnitine and acute paracetamol (acute APAP) treated group compared to acute paracetamol treated group		
	Mean	S.E.M	Mean	S.E.M	% change	Mean	S.E.M	% change	Mean	S.E.M	% change
Urea (mg/dl)	25.75	1.1	24.01	0.5 †	- 7 %	96.75	3.2 *	+ 275.7 %	20.5	0.9 *	- 78.81%
Creatinine (mg/dl)	0.31	0.02	0.30	0.01 †	- 3.2%	0.84	1.5 *	+ 170.9 %	0.26	0.1 *	- 69.04%
GSH (mg/L)	38.69	0.52	38.31	0.3 †	-1%	22.91	0.7 *	- 40.8%	37.21	0. *	+ 62.42%

* Significant, P value <0.05; † Insignificant, P value >0.05

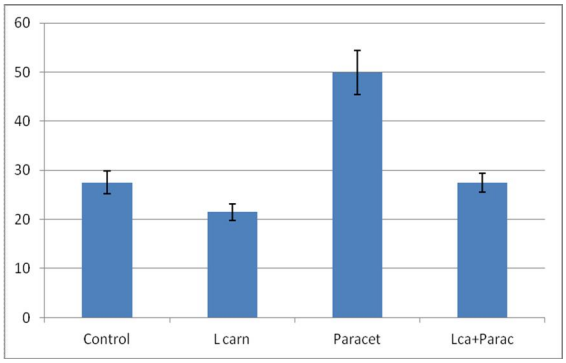
Table 2: Effects of paracetamol and L-Carnitine treatments on renal superoxide dismutase (SOD) and catalase (CAT) levels.

Groups / Parameters	Normal Control group		L-Carnitine treated group compared to normal control group			Acute paracetmol (APAP) group compared to normal control group			L-Carnitine and acute paracetamol (acuteAPAP) treated group compared to acute paracetamol treated group		
	Mean	S.E.M	Mean	S.E.M	% change	Mean	S.E.M	% change	Mean	S.E.M	% change
SOD (u/mg protein)	8.99	0.25	8.4	0.09	- 6.56 % †	3.5	0.15 *	- 61.07 %	6.99	0.2 *	+ 99.7 %
CAT (u/mg protein)	0.40	0.1	0.39	0.1	- 2.5 % †	0.25	0.1 *	- 37.5 %	0.38	0.02 *	+ 52 %

* Significant, P value <0.05; † Insignificant, P value >0.05

Table 3: Mean value of cell necrosis measured as vacuolated areas

	Control	L carn	Paracet	Lca+Parac
Mean	27.55507	21.4769	49.85599033	27.4641074
stdev	79.192	66.94748	200.8355997	66.1914106
StE	2.387729	1.700468	4.477408395	1.94177351
Pc		0.160195	3.68272E-08	0.00904388
pL			3.64643E-09	0.02056365
pP				4.7921E-06



Histogram. 1: Mean values of vacuolated areas representing cell necrosis in control and treated kidney sections. Error bars represent the standard deviation from the mean

Acute APAP group:

Light microscopic examination of the kidney specimens of acute APAP treated animals, showed shrinkage of some glomeruli with dilatation of the capsular space and some glomeruli appeared atrophied with extra vasation as in fig(3).Some of the tubules showed cyptoplamic vacuolation and pyknotic nuclei (Fig.3).

L-Carnitine +Acute APAP group:

Light microscopic examination of this group revealed that the L-Carnitine protected the kidney from the destructive effect of APAP in both the glomeruli and the tubules. Most of the glomeruli were appeared more or less normal in structure and the Bowman's space returned to its normal width (Fig.4A).The tubules showed lessen cyptoplamic vocuolations and dilated lumen of some of them (Fig.4B).

Quantitative evaluation of cell necrosis:

Table (3) and Histogram (1) showed the mean values of vacuolated areas represented cell necrosis in control and treated kidney sections.

There is a histological variation between control and treated groups (Histogram 1). Quantification of necrotic areas in the kidney of control and treated groups (Table 3 and Histogram 1) revealed that there was a significant ($p \leq 3.6x10^{-8}$) increase in necrotic areas in acute APAP treated group (49.8± 4.5) compared to control (27.5± 2.4) or L-carnitin treated (21.5± 1.7) groups.

The use of L-carnitine and paracetemol resulted in values nearing that of control (27± 1.9).

Electron Microscopic Results:

Control Group:

Electron microscopic examination of control group revealed normal ultra structural features of glomerulus was composed of several capillary loops .each capillary lined by endothelial cells with large

nucleus bulged in the capillary lumen resting on trilaminar basement membrane .The normal podocyte with discrete foot processes was attached to glomerulus basement membrane(GBM)(Fig.5A).

The proximal convoluted tubules showed normal apical microvilli, normal rounded nucleus with multiple basal mitochondria (Fig.5B).

The distal convoluted tubules showed normal basal invaginatioun and normal basement membrane (Fig.5C).

L-Carnitine group:

Electron microscopic examination of the glomeruli showed normal GBM thickness and normal width of filtration slits(Fig.6A).The cells of proximal convoluted tubule (PCT) had rounded euchromatic nuclei. The mitochondria showed normal arrangement at the base and interdigitated with the basal membrane infoldings forming the basal striations. The cells showed packed microvilli at its apex (Fig.6A).the cells of distal convoluted tubule had rounded nuclei with few apical microvilli (Fig.6B).

Acute APAP group:

Electron microscopic examination of this group revealed irregular thickness of glomerulus basement membrane with loss of trilaminar appearance, fusion of foot processes of podocytes in some areas and widening of some filtration slits of podocyte (Figs.6 A+B). Some cells of the DCT contained vacuoles of different size and shape, few nuclei were heterochromatic and also pyknotic nuclei appeared in some cells. Some tubules lost basal striation and mitochondria lost their pattern of basal arrangement. Massive extra tubular collagenous fibers were observed around the tubules in the extra tubular region (Figs.7 A+B +C +D).

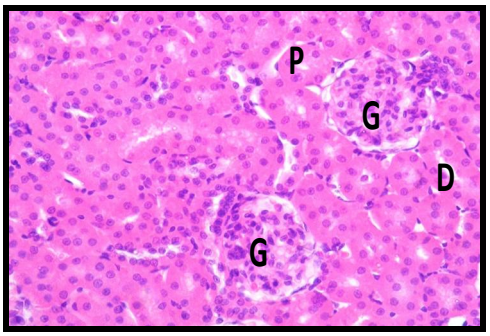


Fig 1: A Section of a kidney of control group 1 showing renal corpuscels demonstrating a normal glomerulus (G) with normal Bowman's Capsule.
Note: proximal (P) and distal (D) convulated tubules.

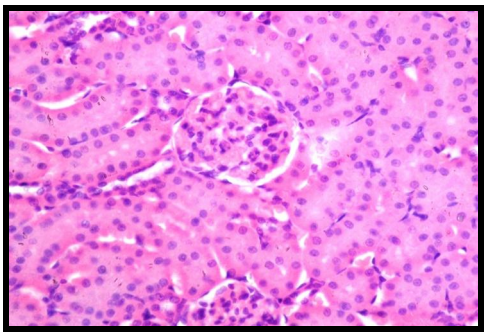


Fig. 2: A Section of L .carnitine treated (group II) showing normal structure of renal corpuscles and tubules .

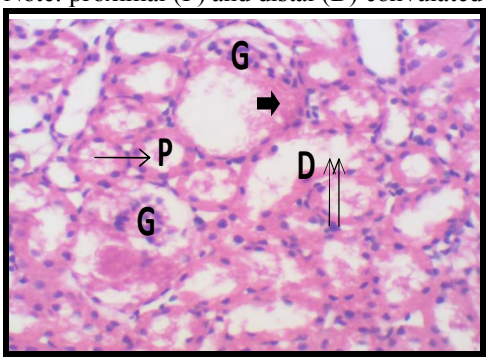


Fig. 3: A Section of acute APAP treated group) group III) showing loss of archtiecture of renal corpuscles with shrinking of some glomeruli (G) and widening of capsular space around it. Extravasations of blood with atrophy of some glomeruli(➡). Note : dilatation of some proximal (↑) & distal tubules (↑↑). Note vacuolation, loss of brush border and shedding of lining epithelium in the lumen of the tubules. (P & D).

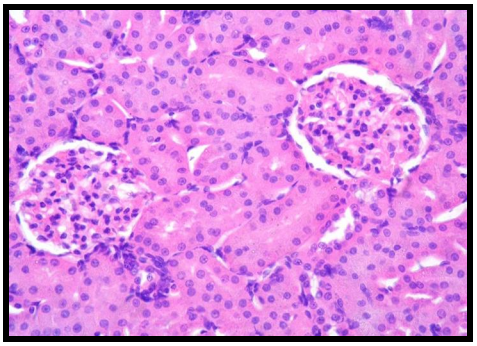


Fig. 4 (A): A section of L.Carnitine and acuteAPAP group(Iv) showing, more or less normal appreance of glomeruli and tubules.

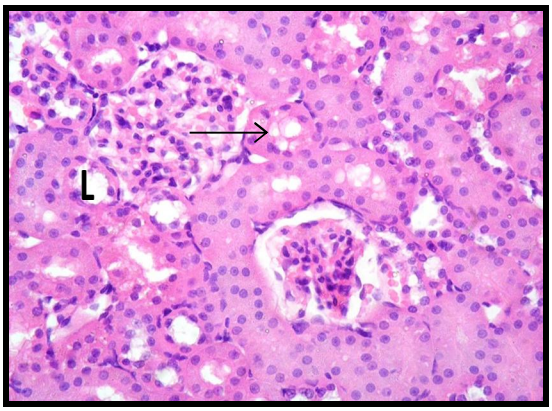


Fig. 4:(B): There are cytoplasmic vacuolation (↙) and mild dilatation in some lumen of the tubules (L).

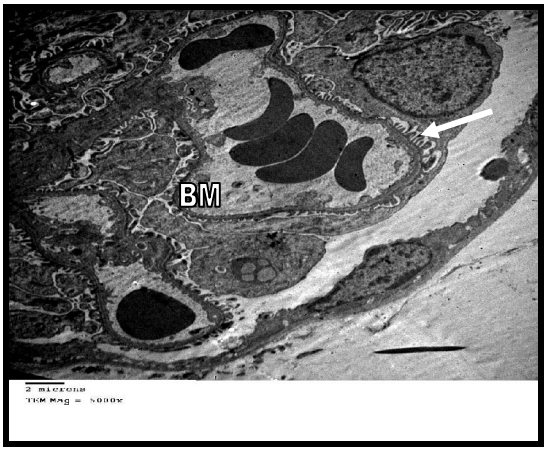


Fig. (5-A):- An ultrathin section of renal cortex of a control rat group (I) showing part of normal glomerulus with normal trilaminar appearance of basement membrane (BM) with uniform thickness And discrete foot processes of podocytes (arrows)
(x 8000)

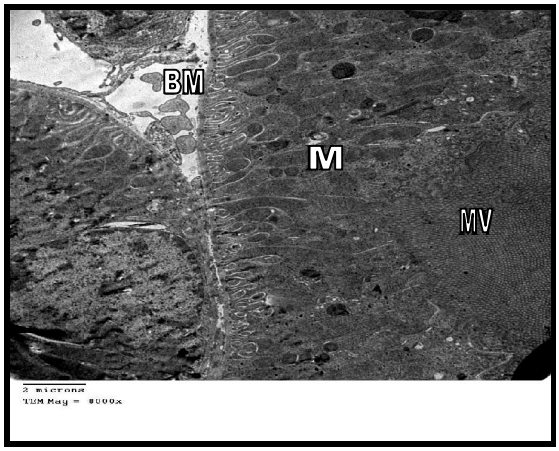
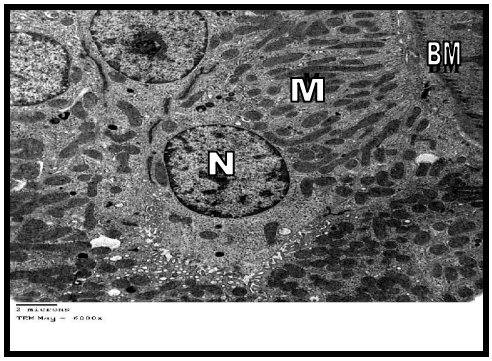


Fig. (5-B):- An ultrathin section of renal cortex of a control rat group (I) showing part of proximal convoluted tubule has apical microvilli (MV) ,basal in folding angle plenty of mitochondria (M) And normal basement membrane (BM).
(x 8000)



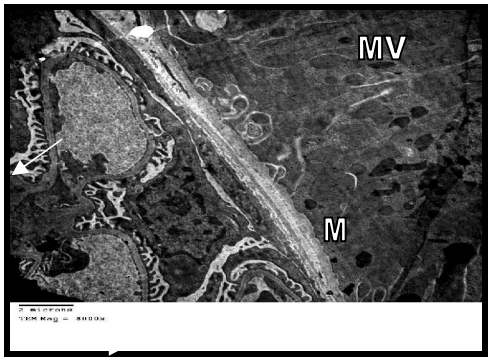


Fig. (5-C):- An ultrathin section of renal cortex of a control rat group (I) showing part of distal convoluted tubule revealing basal invagination, mitochondria (M) round nuclei (N) and normal basement membrane (BM).
(x 8000)

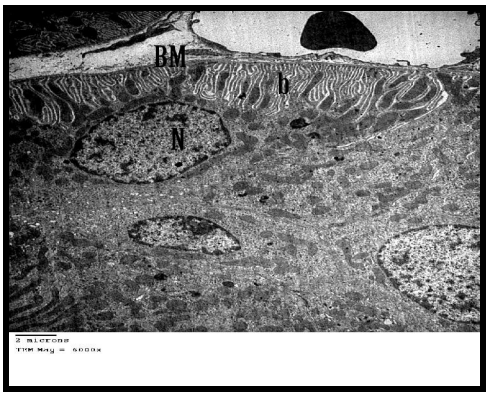


Fig. (6-B):An ultrathin section of renal cortex of L- carnitine treated group (II) showing a part of a distal convoluted tubule revealing basal infolding (b) mitochondria normal central nuclei (N) and normal basal membrane (BM) . (x 6000)

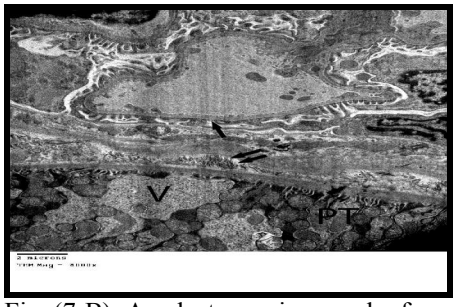


Fig. (7-B) :An electron micrograph of an ultra thin section of a renal cortex of acute APAP group III showing , widening of filtration silts(↑) in some area. presence of massive Collagenous (↑↑) fibers around tubules. Loss of mitochondrial and basal in folding pattern, presence of non membrance cytoplasmic vacuoles (V) of different sizes in proximal tubule (PT). (x 8000)

Fig. (6-A): An ultrathin section of renal cortex of L- carnitine treated group (II)showing a part of renal corpuscle demonstrating normal structure of glomerules with normal thickness of glomerular basement membrane (↑↑) and normal infiltration slits (↑) Notes a part of proximal convoluted tubules revealing of numerous apical microvilli (MV) and multiple mitochondria (m) in between basal infolding.
(x 8000)

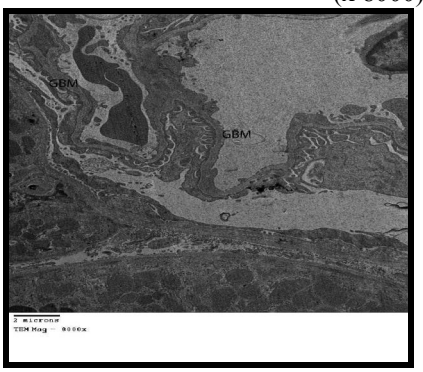


Fig. (7- A): An ultra thin section of a renal cortex of acute APAP group (III) showing increase in thickness of GBM with loss of its trilaminar appearance and fusion of foot processes of podocytes. (x 8000)

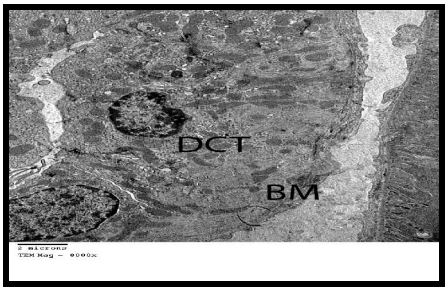


Fig. (7-C): An ultra thin section of a renal cortex of acute APAP group III showing one cell shows small heterochromatic nuclear and thickening of BM of distal convoluted tubules (DCT). (x 8000)

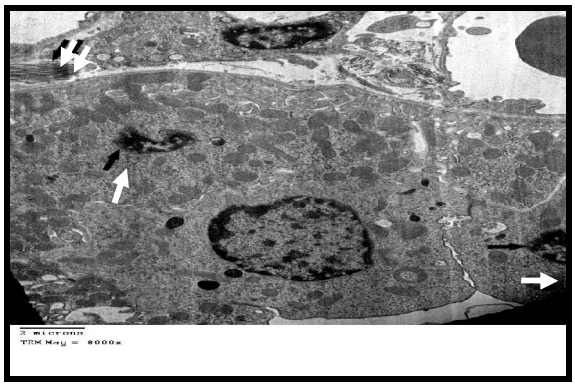


Fig. (7-D): An ultra thin section of a renal cortex of acute APAP group (III) showing pyknotic nuclei in cells of distal convoluted tubule. (↑) Note extra tubular collagenous fibers (↑↑). (x 8000)

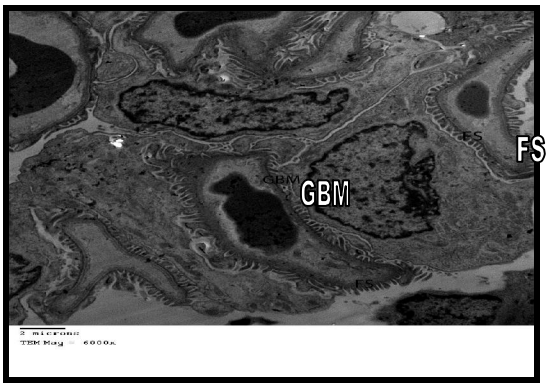


Fig. (8-A): An ultra thin section of renal cortex of L.carnitine + acute APAP of group (IV) showing: a part of glomerulus with more or less normal structure. Notice normal width of the filtration slits (FS) with normal thickness of the glomerulus basement membrane (GBM). (X6000)

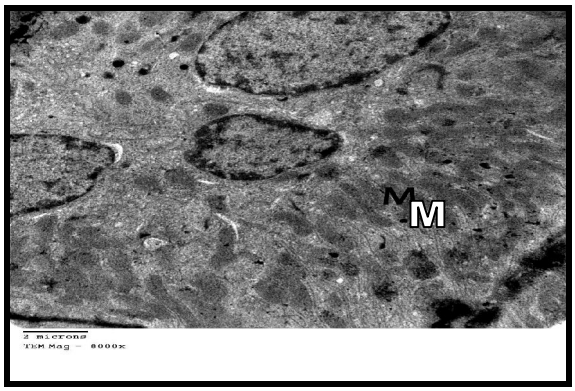


Fig (8-B): An ultra thin section of renal cortex of L.carnitine + acute APAP of group (IV) showing a part of a distal convoluted tubule demonstrating normal basal in folding and mitochondria (M). (x 8000)

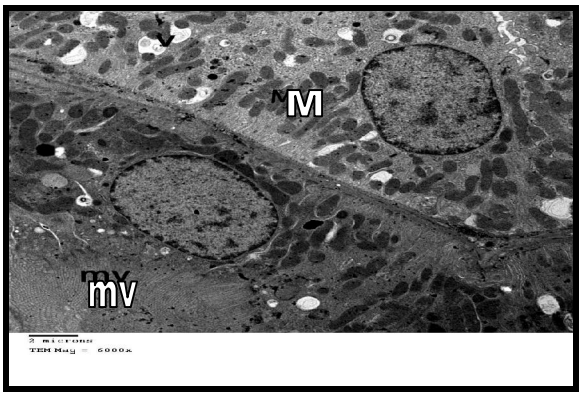


Fig. (8-C): An ultra thin section of renal cortex of L.carnitine + acute APAP of group (IV) showing, a part of proximal convoluted tubule with normal appearance of mitochondria (M), basal in foldings. and normal apical microvilli (mv). Few collagen fibers in extra tubular region and perance of cytoplasmic vacuoles (V). (x 6000)

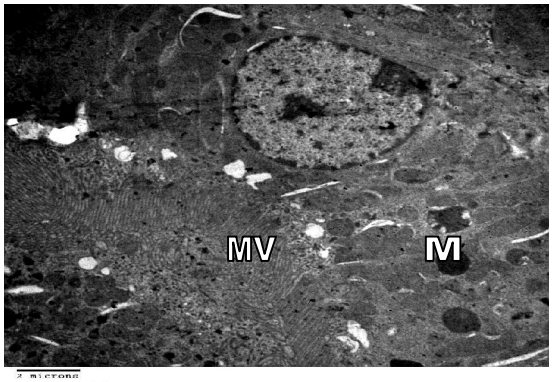


Fig. (8-D): An ultra thin section of renal cortex of L.carnitine + acute APAP of group (IV) showing, a part of proximal convoluted tubule with normal appearance of mitochondria (M), and normal apical microvilli (mv). (X 8000)

L-Carnitine +Acute APAP group:

Electron microscopic examination of this group showed more or less normal ultra structure of the glomerulus , there were no obvious thickening of the glomerular basement membrane, the filtration slits regained its normal width (Fig.8A). DCT demonstrated normal basal infolding and normal mitochondria (Fig.8B).PCT cells regained their normal structure with few vacuoles .The tall packed microvilli and the mitochondria regained their normal architecture of basal arrangement and intermingled with the basal membrane infoldings and all nuclei showed euchromatic chromatin (Fig.8C & D). Few collagen fibers were observed in the extra tubular region (Figs.8C).

4. Discussion

Paracetamal over dose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine dearrangements. Increased concentrations of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animal and man.

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. On the other hand, creatinine is mostly derived from endogenous sources by tissue creatinine breakdown. Thus serum urea and creatinine are considered as nephrotoxicity markers, but serum urea concentration is often reliable renal function predictor than serum creatinine *(*(Palani *et al*., 2009*)*.

In the present study, administration of nephrotoxic dose of paracetamal to rats resulted in significant elevation of serum levels of urea and creatinine in paracetamol administrated group when compared to the normal control group and L-Carnitine group.

The present results are consistent with that observed by Isik *et al.* (2006*)* who observed an elevation in serum urea and creatinine in rats after 1 g/kg b.w of paracetamol administration. Also, Satirapoj *et al.* (2009*)* reported an elevation in serum urea and creatinine in a woman following therapeutic dose of paracetamal 3 days before hospital admission.

The same observations noticed by Boutis and Shannon (2001*)* in children who were admitted at a tertiary care children's hospital for treatment of severe acute paracetamal intoxication with high levels of serum urea and creatinine.

The histological finding in paracetamol treated group by light and electron microscopic examinations showed changes in renal cortex especially in glomeruli, proximal and distal convoluted tubules. The glomeruli revealed thickening of the glomerular basement membrane, widening of the filtration slits. The proximal convoluted tubules (PCT) lost some of their mitochondrial and basal infolding pattern with presence of cytoplasmic vacuolation. Furthermore, acute paracetamol administration resulted in thickening of their basement membranes and increase in collagen deposition around the tubules in addition to the macrophages which have been shown to contribute to the development of renal fibrosis. These changes explain the manifested impairment in renal functions. The alteration in glomerular structure, thickening of the glomerulus basement membrane, may decrease the glomerular filtration rate and hence retention of toxins in the circulation and hence lead to elevation of serum urea and creatinine. The tubular affection which was observed by the present histological study could be another explanation for the increased serum urea and creatinine. Since there is a part of body creatinine and other body toxins excreted via the convoluted tubules (Marieb, 2006*)*. The tubular destruction can cause retention of these toxins including urea and creatinine and elevation of their serum levels.

The previous histological changes were confirmed by Li *et al.* (2003) and Abraham *et al*.(2005*),* they reported that single administration of 1g/Kg b.wt of paracetamol could produce tubular epithelial cells degeneration, vacuolization and desquamation with partial occlusion of tubular lumen by cellular debris. Also, Stern *et al. (*2005*)* observed tubular necrosis after one day of 0.4g/kg b.w. of paracetamol administration to male albino rats. Sener *et al. (*2005) reported that 0.9 g/kg of paracetamol as acute single dose caused degeneration of both the tubules and glomeruli associated with cellular debris in the PCT with increased collagen contents in the extratubular region. Abdel-Zaher *et al.,* (2007*)* stated that acute oral toxic dose of paracetamol 2.5 g/kg causes moderate cloudy swelling of proximal convoluted tubules and severe vacuolar degeneration of distal tubules.

Several studies have clearly demonstrated that, acute paracetamol overdose induced renal oxidative stress as manifested by a decrease in antioxidant enzymes with an increase in lipid peroxidation product (malondialdehyde) *(Chen et al*., 2000*; Abdel* – Zaher *et al* ., 2007and Ghosh and Sil, 2007*)*.

The present study demonstrated that, the administration of paracetamol (APAP) in acute single toxic dose resulted in a significant decrease in renal SOD and CAT activities when compared to control group. The present results are in agreement with that observed by Palani *et al. (*2009 *)and* Demirbag*, and Uysal* .(2010*)* who observed a significant decrease in levels of SOD and CAT after paracetamol overdose

administration to rats when compared with normal control rats and explained these results on the base that paracetamol overdose enhances lipid peroxidation or inactivates the antioxidative enzymes.

In addition, Linares *et al. (*2006*)* reported that, during kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism.

Current evidence suggests that, intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney *(*Richie et al., 1992 and Newton *et al.,* 1996*)*. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in paracetamol toxicity *(*Manov *et al.,* 2003*)*.

Paracetamol overdose administration also caused a significant decrease in serum GSH content in the present study. This decrease in GSH level could be considered another mechanism for the observed paracetamol nephrotoxicity and explained as the administration of high dose of paracetamol saturate the metabolic pathway decreasing the liver clearance of APAP and allows higher amounts of the unmetabolized drug to come in contact with the kidney *(*Gu *et al.,* 2005).Because the same enzyme system as the liver are also present in the kidney, it is most probable that NAPQI will also be formed in the kidneys giving rise to toxicity, but much later than in the liver (Roberts and Bukley, 2007*)*. The major pathway of metabolism during toxicity is via CYP-450 due to saturation of glucurinidation and sulfation pathways forming the intermediate NAPQI in high amounts which in turn will be conjugated with GSH to detoxify this product with consequent exhaustion of cellular GSH reserve *(*Sener *et al.,* 2005*)*.

At sufficiently high doses, GSH becomes depleted leaving NAPQI free *(*Dai *et al*., 2006*)*. The free NAPQI is strongly electrophilic and bind covalently and irreversibly to critical cellular protein causing cellular necrosis (James *et al.,* 2003*)*.

The depletion in GSH level after acute paracetamol overdose is consistent with the observation of Chen *et al.* (2000*)* who reported that the use of parcetamol overdose (0.375 g/ kg/ b. wt) in one shot resulted in significant decrease in renal cortical GSH in old mice. This depletion of GSH is also accompanied by enhanced production of reactive oxygen species resulted in alteration of the glomerular basement membrane.

Other histological changes included heterochromatic nucleus which was observed in the electron microscopic findings of the proximal convoluted tubules which was one of the apoptotic manifestation in all cells. These changes came in agreement with Lorz *et al*. (2004*)* who described apoptosis in proximal convoluted tubules as another mechanism of acute paracetamol induced nephrotoxicity.

Cytoplasmic vacuolation was noticed in the present study in proximal and distal convoluted tubules owing to the changes in ions and water movement through the membranes as reported in toxic tubular necrosis. Robbins *et al*.(1984) stated that such cellular vacuolation was most probably a cellular defense mechanism against injurious substances in which these substances were segregated in the vacuoles and were thus preventing the interference with cellular metabolism .

In the current research, there was focal thickening, wrinkling and loss of trilamellar appearance of glomerular basement membrane that might be due to repeated damage of the epithelial cells with regeneration and secretion of new material. However, the increased thickening in the glomerular and tubular basement membrane was attributed to the increased deposition of glycoproteins (Hotta *et al.,* 2001). Fusion of foot processes of the podocytes were observed in the present study and were suggested by many previous researches to be related to proteinuria which was caused by leakage of protein through the distorted basement membrane indicating glomerular damage(Gabbai, 1995*)*.Thus the fusion of foot processes and increased thickening of the glomerular basement membrane compensated for increased permeability and proteinurea.

Interestingly, the present study revealed that previous administration of L-Carnitine 500 mg/kg b.wt for 7 days before acute paracetamol overdose administration resulted in improvement of the kidney functions in the form of elevation of the oxidative stress enzymes SOD, CAT and GSH enzyme activities and decrease serum levels of urea and creatinine. These findings were in agreement with Aydogdu *et al.* (2006*)* who reported that, L-Carnitine which is an antioxidant and prevents the accumulation of end products of lipid peroxidation when injected at a dose 200 mg/kg i.p. can reduce the elevated levels of serum urea and creatinine and elevate the level of antioxidant enzymes SOD, CAT and GSH activities. They explained these changes as that the acute paracetamol overdose induced marked renal oxidative stress and renal damage which was manifested by the increased serum level of urea, creatinine and glutathione with decrease the kidney tissue superoxide dismutase (SOD) and catalas

(CAT) levels. All these changes were significantly improved by L- Carnitine supplementation, which indicating that L- carnitine treatment protects against functional, biochemical and morphological damage in rats.

The present results are also consistent with Boonsanit *et al*. (2006*)*. They reported that, 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 were related to the improvement of glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and decrease the renal vascular resistance (RVR) induced by L- Carnitine treatment.

The biochemical results were also confirmed by the histological findings which showed normal width of the filtration sites, decrease in the glomerular basement membrane thickness when compared to paracetamol treated group. Also the foot processes of the podocytes were intact with normal tall packed microvilli and normal pattern of mitochondria associated with less cytoplasmic vacuoles and less thickening of the basement membrane of the PCT. Also, the extratubular collagen was less manifested when compared to paracetamol treated group.

These histological observations were confirmed by Tufekci *et al.* (2009*)* who observed an improvement in glomerular conditions in the form of decreased thickening of the glomerular basement membrane and intact foot processes of podocytes by the electron microscope examination when L-Carnitine is used as a protective agent against cisplastin induced nephrotoxicity.

In conclusion, antioxidative and antiapoptotic properties of L-Carnitine were supported in the present study by the findings that this agent improves kidney function tests and has a protective role via inhibition of apoptosis in L-Carnitine induced nephrotoxicity.

Corresponding author
Zeinab Mahmoud Gebaly
Histology, Departments, Faculty of Medicine for girls, Al-Azhar University
zienabgebaly@live.com

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