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# Research Article
# The Influence of L-carnitine on Aspartame Toxicity in Kidney of Male Rats

[1]Rasha A. Al-Eisa, [1,2]Reham Z. Hamza, [3]Amir E. Mehana and [3]Nahla S. El-Shenawy

[1]Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia
[2]Department of Zoology, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt
[3]Department of Zoology, Faculty of Science, Suez Canal University, Ismailia, 41522, Egypt

## Abstract

**Background and Objective:** Aspartame (ASP) one of the famous artificial sweeteners used as substitution of the sugar in foods and beverages. The study was aimed to investigate the oxidative responses and histopathological changes induced by ASP on the kidney of rats and the ameliorative role of L-carnitine (LC) to prevent the toxicity. **Materials and Methods:** Rats were split into six groups (n = 8) as follow: control, ASP (low dose, LD) (75 mg $kg^{-1}$), ASP (high dose, HD) (150 mg $kg^{-1}$), 10 mg $kg^{-1}$ of LC, ASP-LD+LC and ASP-HD+LC, all groups treated for successive 30 days. **Results:** The ASP marked decreased the renal levels of reduced glutathione (GSH), activities of antioxidant enzyme markers and increased lipid peroxidation levels. DNA damage was significantly increased in ASP-LD and ASP-HD groups as compared to control animals. The LC prevented the ASP-induced kidney damage as specified by ameliorating all the above-mentioned parameters. Histopathological changes were parallel with the biochemical alternation in ASP groups. **Conclusion:** The renal toxicity induced by ASP in rats could be improved by LC through different protective mechanisms.

Key words: Artificial sweeteners, aspartame, L-carnitine, lipid peroxidation, renal toxicity

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Corresponding Authors: Nahla S. El-Shenawy, Department of Zoology, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt
Tel: 002/01008660620
Reham Z. Hamza, Department of Zoology, Faculty of Science, Zagazig University, Zagazig 44519, Egypt Tel: 01274780563, 00966531355470



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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Aspartame (L-α-aspartyl-L-phenylalanine, 1-methyl ester) is used exceedingly, as synthetic sweeteners among a diversity of foods (chewing gum, soft drinks, etc.) and hygiene products[1]. It is used by diabetic and human those need to lose weight and metabolized after administration to phenylalanine, aspartic acid and methanol[2]. The latter is oxidized to formaldehyde and subsequently oxidized into formic acid and NADH as well as the formation of superoxide anions, that significant increase the lipid peroxidation (LPO).

L-carnitine (γ-three methyl amino-β-hydroxyl fatty acid, LC) is very important in fatty acids transfer from the cytosol to mitochondria[3]. Moreover, LC has a preservative effect on LPO by rebating the formation of $H_2O_2$ and it has the ability to remove the superoxide radical[4,5]. The LC can be taken from food or is assembled endogenously from two amino acids, methionine and lysine, which are essential in tissues such as skeletal muscles, heart, brain, kidney and liver [6]. It has been found to improve anemia and thrombocytopenia as well as reducing the oxidative stress in the heart of oophorectomized rats[7].

The kidney is one of the organs responsible for maintaining extracellular environment constant by excretion of such purine catabolites as urea, creatinine and uric acid. Increasing the levels of these catabolites used as renal function impairment[2]. It is susceptible to the toxic substances as it is one of the main organs of excretion. Therefore, the current study was proceeded to evaluate the potential ameliorative influences of LC opposed to nephritic toxicity elicited by ASP in male rats. The oxidative and the histological changes of kidney tissues were assessment. The genotoxicity data was determined using comet assay to evaluate the possible DNA injury induced by ASP.

## MATERIALS AND METHODS

**Chemicals:** Aspartame (ASP) was brought from Sigma-Aldrich chemical; St. Louis, USA. Other chemical were of analytical grade and obtained from Sisco Laboratory, India.

**Animal and experimental design:** The experimental animals were healthy adult male Wistar albino rats, weighing approximately 200-250 g and were obtained from the Faculty of Veterinary Medicine at Zagazig University. The animals were preserved under standard laboratory circumstances and were allowed to standard rat feed pellets *ad libitum*. Animal experiments were performed after following the European Community Directive (86/609/EEC) and national rules in accordance with the 8th edition of NIH Guidelines for the Use of Animals. Animal experiments received approval from the Ethical Committee of Pharmacy Faculty of Zagazig University (No. P22/2/2013).

The rats were divided into six groups (n = 8). Group I was served as the control animals and was given saline as a vehicle. Group II and group III were treated with low dose of ASP (ASP-LD) and the high dose of ASP (ASP-HD) (75 and 150 mg $kg^{-1}$, respectively)[8]. Group IV was given with LC (10 mg $kg^{-1}$)[8]. Groups V and VI were administrated ASP-LD+LC and ASP-HD+LC, consequently. All the animals were given treatment orally for 30 successive days.

**Blood collection and renal biomarkers evaluation:** The samples of blood were harvested from overnight fasted animals by retro-orbital puncture under anesthesia with ether. Samples were centrifuged at 4000 rpm for 15 min. Serum was harvested and preserved at -80°C until kidney biomarkers estimated.

Creatinine and uric acid were estimated according to Schirmeister *et al.*[9] and Fossati *et al.*[10]. The urea level was estimated according to Patton and Crouch[11].

**Preparation of kidney homogenates and oxidative response estimation:** Kidney tissues were used for oxidative stress analysis and determination of antioxidant parameters. Tissues were embedded with a 50 mM of sodium phosphate buffer (pH 7.4), 0.1 mM EDTA and 0.25 M sucrose. After centrifugation, the supernatants were modulated into Eppendorf and stored at -20°C until being used for further measurements.

In the kidney tissue, LPO was estimated based on the release of malondialdehyde (MDA) as the marker of LPO[12]. Superoxide dismutase (SOD) and catalase (CAT) were estimated by Marklund and Marklund[13] and Aebi[14], respectively. The glutathione peroxidase (GPx) activity was determined[15]. The activity of GPx was expressed in terms of mol GSH consumed/min/g wet weight tissue.

Glutathione reduced (GSH) level in the tissue was determined by Beutler *et al.*[16]. Serum myeloperoxidase (MPO) and xanthine oxidase (XO) activities were estimated[17,18].

**Single cell gel electrophoresis (SCGE):** Pieces of the kidney of the control and treated groups were put into an ice-cold Petri dish ($Ca^{2+}$ and $Mg^{2+}$ free HBSS containing 10% DMSO and 20 mM EDTA). The cells vitality was estimated indirectly by analyzing the comet images after electrophoresis[19].

**Histological evaluation:** Samples of kidney were taken from all rat groups and fixed in 10% formal saline for 24 h. The sections were stained by eosin and hematoxylin for examination through the light microscope and using a digital camera for photographing[20].

**Statistical analysis:** Data are expressed as a Mean±Standard Error (SE). All data were analyzed with the SPSS for Windows statistical package by using One Way-Analysis of Variance (ANOVA).

## RESULTS

Uric acid, urea and creatinine were estimated to recognize the nephritic functions (Table 1). The marked increment levels of all parameters in ASP treated groups at all tested doses were observed. The elevation of urea level was observed by doses increment to 1.5 and 1.8-fold incomparable to control for ASP-LD and ASP-HD, respectively. The uric acid levels were increased by 1.7 and 2.1-fold in ASP-LD and ASP-HD in comparison to control, respectively. Treatment the rats with ASP-LD and ASP-HD elevated the creatinine levels to 3.5 and 4.5-fold in comparison to control, respectively. The total bilirubin was elevated in a dose-dependent way as the effect of ASP. Treatment the mice with LC and ASP ameliorated the level of nephron-functions parameters markedly incomparable with each its relative ASP-group.

Alterations in antioxidant enzymes of kidney and thiol level of male rats treated with ASP or/and LC were presented in Table 2. Serum MPO and XO activities were elevated as the effect of ASP while the thiol level decreased. They were reinforced when rats administrated with LC and ASP.

The results proved LPO increase in the kidney of the ASP-treated group in a manner depending on the dose as manifested by the elevated malondialdehyde levels in kidneys tissue (Fig. 1). The administration of LC before ASP mitigated LPO stimulated by DM treatment and significantly depleted the MDA levels in the renal tissue.

The SOD, CAT and GPx activities decreased markedly in ASP groups when compared to controls (Fig. 2-4). Administration of LC with ASP ameliorated the antioxidant enzymes as compared to ASP-group.

A significant decrement of GSH and non-protein thiol (NPSH) levels of renal tissue was evident in ASP-group incomparable to the controls (Fig. 5, Table 2). Concurrent treatment of LC with ASP to the rats ameliorated GSH and NPSH levels when compared to ASP-groups.

The results of the comet assay are shown in Table 3 and Fig. 7 as the percent tail DNA and tail moment of the kidney.

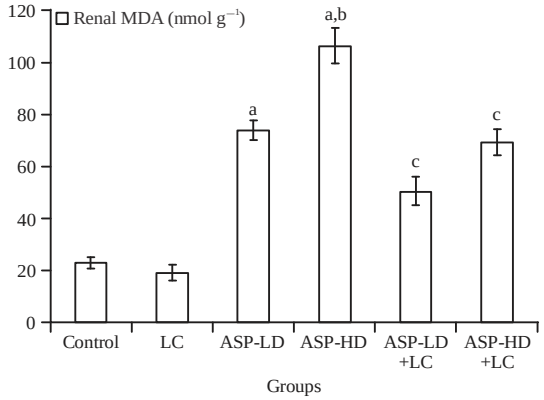


Fig. 1: Changes in renal lipid peroxidation (MDA) levels in male rats treated with aspartame or/and L-carnitine. Values are expressed as Mean±SE, n = 8 for each treatment group. [a]Significant difference as compared to control, [b]Significant difference as compared to aspartame-low dose group (ASP-LD) and [c]Significant difference as compared to its relative group of ASP
LC: L-carnitine, ASP-LD: The low dose of aspartame and ASP-HD: High dose of aspartame

Table 1: Changes in renal functions of male albino rats treated with aspartame or/and L-carnitine

| Parameters | Control | LC | ASP-LD | ASP-HD | ASP-LD and LC | ASP-HD and LC |
|---|---|---|---|---|---|---|
| Urea (g $dL^{-1}$) | 21.48±0.49 | 20.15±0.46 | 32.53±1.35[a] | 38.41±1.18[a,b] | 23.78±1.27[c] | 25.26±1.03[c] |
| Uric acid (g $dL^{-1}$) | 14.09±1.28 | 15.18±0.69 | 24.12±1.50[a] | 29.30±1.54[a,b] | 16.43±0.71[c] | 18.20±1.26[c] |
| Creatinine (g $dL^{-1}$) | 0.56±0.09 | 0.52±0.03 | 1.97±0.14[a] | 2.51±0.22[a] | 0.86±0.06[c] | 1.01±0.08[c] |

Values are expressed as Mean±SE, n = 8 for each treatment group, LC: L-carnitine, ASP-LD: Low dose of aspartame, ASP-HD: High dose of aspartame, [a]Significant difference as compared to control, [b]Significant difference as compared to ASP-LD and [c]Significant difference as compared to its relative group of ASP ($p \leq 0.05$)

Table 2: Changes in serum renal antioxidant enzymes and thiol level in male rats treated with aspartame or/and L-carnitine

| Parameters | Control | LC | ASP-LD | ASP-HD | ASP-LD and LC | ASP-HD and LC |
|---|---|---|---|---|---|---|
| Renal myeloperoxidase (MPO) (nmol/min/mL) | 22.80±0.91 | 24.13±1.75 | 34.74±0.54[a] | 41.14±0.78[a,b] | 27.68±1.02[c] | 28.58±1.84[c] |
| Renal xanthine oxidase (XO) (U $g^{-1}$) | 15.57±1.01 | 16.41±0.74 | 25.09±1.02[a] | 31.29±1.23[a,b] | 21.26±1.15[c] | 21.96±1.90[c] |
| Renal thiol (μmol $g^{-1}$) | 14.82±0.45 | 16.27±0.42 | 5.76±0.52[a] | 5.15±1.60[a] | 12.33±1.26[c] | 11.84±2.28[c] |

Values are expressed as Mean±SE, n = 8 for each treatment group. LC: L-carnitine, ASP-LD: Low dose of aspartame and ASP-HD: High dose of aspartame. [a]Significant difference as compared to control, [b]Significant difference as compared to ASP-LD and [c]Significant difference as compared to its relative group of ASP ($p \leq 0.05$)

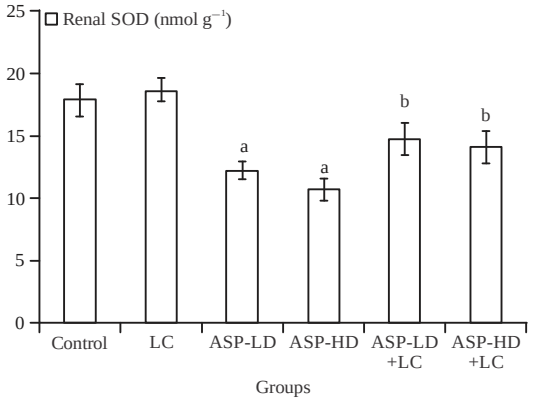


Fig. 2: Changes in renal superoxide dismutase (SOD) activity in male rats treated with aspartame or/and L-carnitine. Values are expressed as Mean±SE, n = 8 for each treatment group

LC: L-carnitine, ASP-LD: The low dose of aspartame and ASP-HD: High dose of aspartame. [a]Significant difference as compared to control and [b]Significant difference as compared to its relative group of ASP

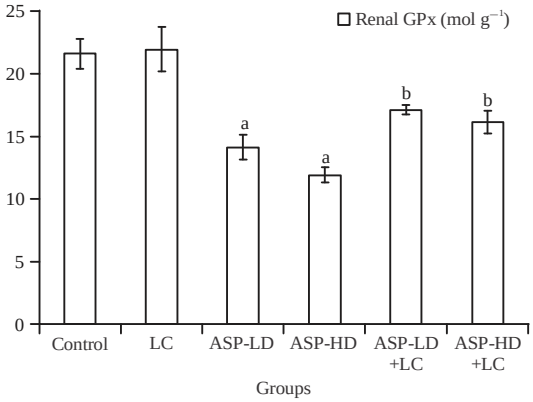


Fig. 4: Changes in renal glutathione peroxidase (GPx) activity of male rats treated with aspartame or/and L-carnitine. Values are expressed as Mean±SE, n = 8 for each treatment group

LC: L-carnitine, ASP-LD: The low dose of aspartame and ASP-HD: High dose of aspartame. [a]Significant difference as compared to control and [b]Significant difference as compared to its relative group of ASP

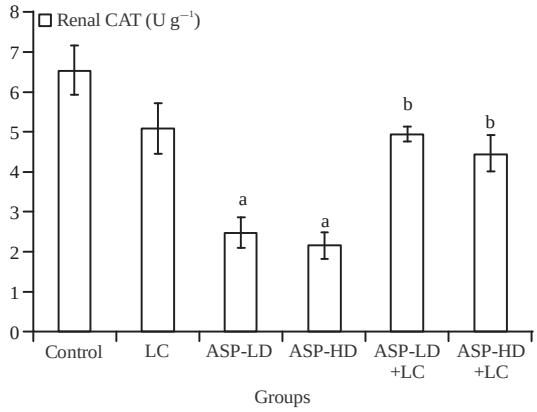


Fig. 3: Changes in renal catalase (CAT) activity of male rats treated with aspartame or/and L-carnitine. Values are expressed as Mean±SE, n = 8 for each treatment group

LC: L-carnitine, ASP-LD: Low dose of aspartame and ASP-HD: High dose of aspartame. [a]Significant difference as compared to control [b]Significant difference as compared to its relative group of ASP

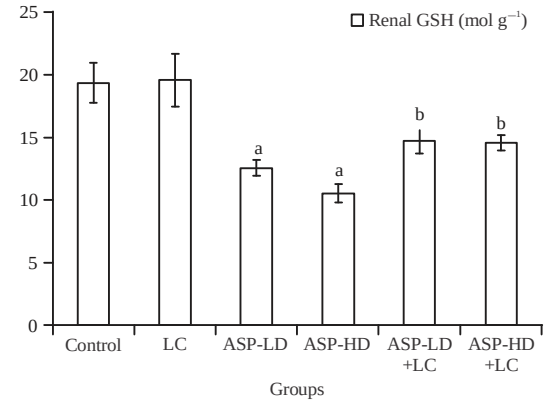


Fig. 5: Changes in renal glutathione (GSH) levels in male rats treated with aspartame or/and L-carnitine. Values are expressed as Mean±SE, n = 8 for each treatment group

LC: L-carnitine, ASP-LD: The low dose of aspartame and ASP-HD: High dose of aspartame. [a]Significant difference as compared to control and [b]Significant difference as compared to its relative group of ASP

The significant and more DNA injury were noticed after ASP-HD treatment. Data declared significant improvement in DNA when the rats treated with LC and ASP at the same time.

Comet images of cells derived from the renal tissue of normal and treated rats (Fig. 6). Control and LC animals showed intact nuclei and normal round cell without a tail (Fig. 6a, b) respectively. ASP-LD group showed damaged DNA strand breaks with low percentage (Fig. 6c), ASP-HD which showed high degree of damage with appearance of and damage nuclei appeared as the cell contains a head like comet and with tail appear as hollow area (Fig. 6d), ASP-LD+LC treated rats showed amelioration of the cells as recorded fewer parameters in the tail length and percentage of damaged DNA and tail and appearance of some intact nuclei (Fig. 6e). ASP-HD+LC group showed intact cells with undamaged DNA and fewer numbers of comet cells (Fig. 6f).

Light microscopic examination of the kidney in the controls and rats that treated with LC (Fig. 7a, b) respectively indicated a normal structure of the renal cortex and tubules.

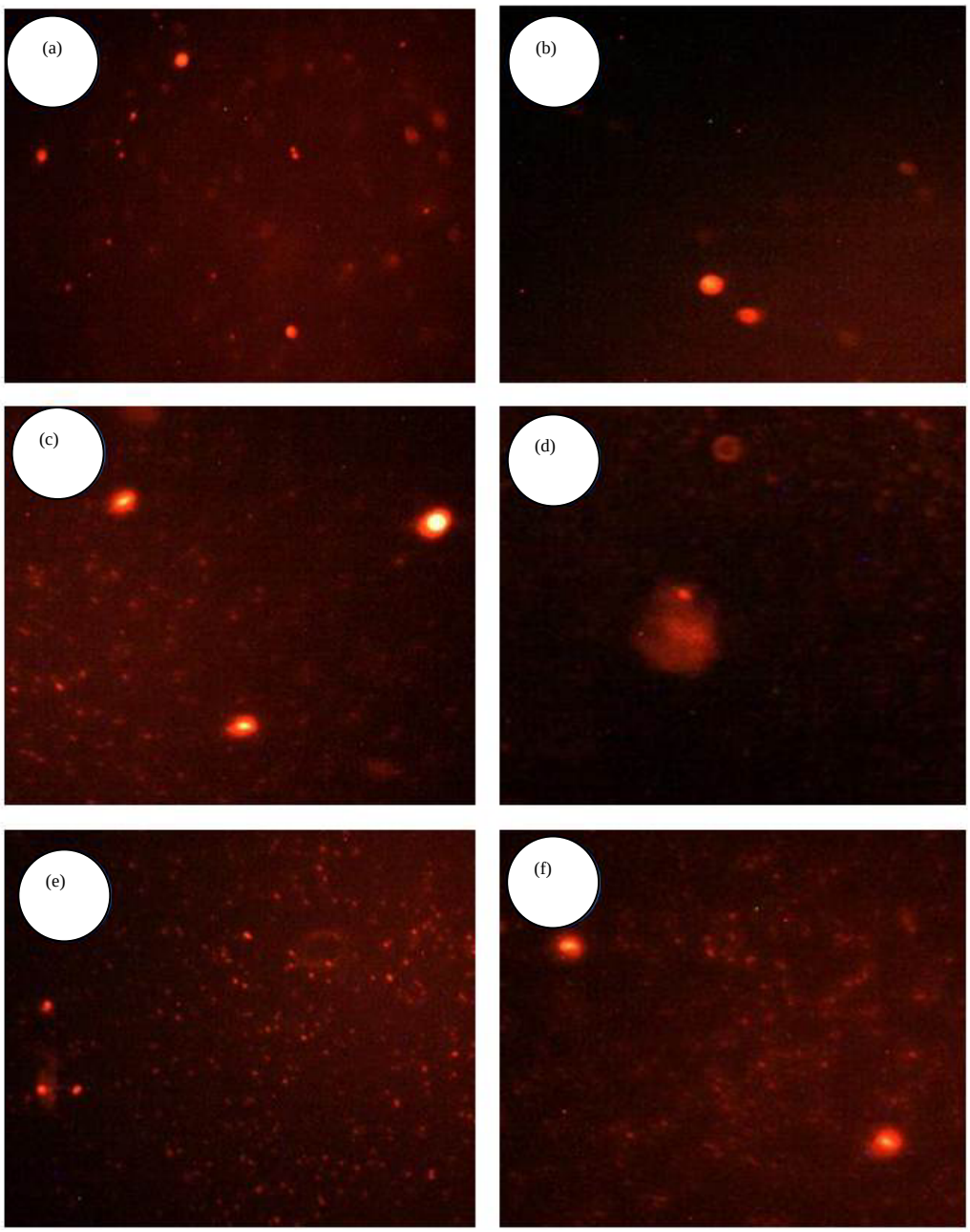


Fig. 6 (a-f): Comet images of cells derived from the kidney of the rat (a) Control group which showed intact nuclei and normal round cell without tail, (b) L-carnitine group which showed intact nuclei with undamaged DNA in a supercoiled state, (c) ASP-LD group showed damaged DNA strand breaks with low percentage, (d) ASP-HD which showed high degree of damage with appearance of and damage nuclei appeared as the cell contains a head like comet and with tail appear as hallow area, (e) ASP-LD+LC which showed amelioration of the cells as recorded fewer parameters in the tail length and percentage of damaged DNA and tail and appearance of some intact nuclei and (f) ASP-HD+LC showed intact cells with undamaged DNA and fewer numbers of comet cells

Control groups of rat showing normal proximal and distal renal tubules (Fig. 7a). Moreover, the cross-section of rat kidney treated with LC showing normal renal corpuscles which consisted of glomeruli encompassed by Bowman's spaces. The proximal convoluted tubules filled by acidophilic cuboidal epithelium with normal brush borders and a narrow

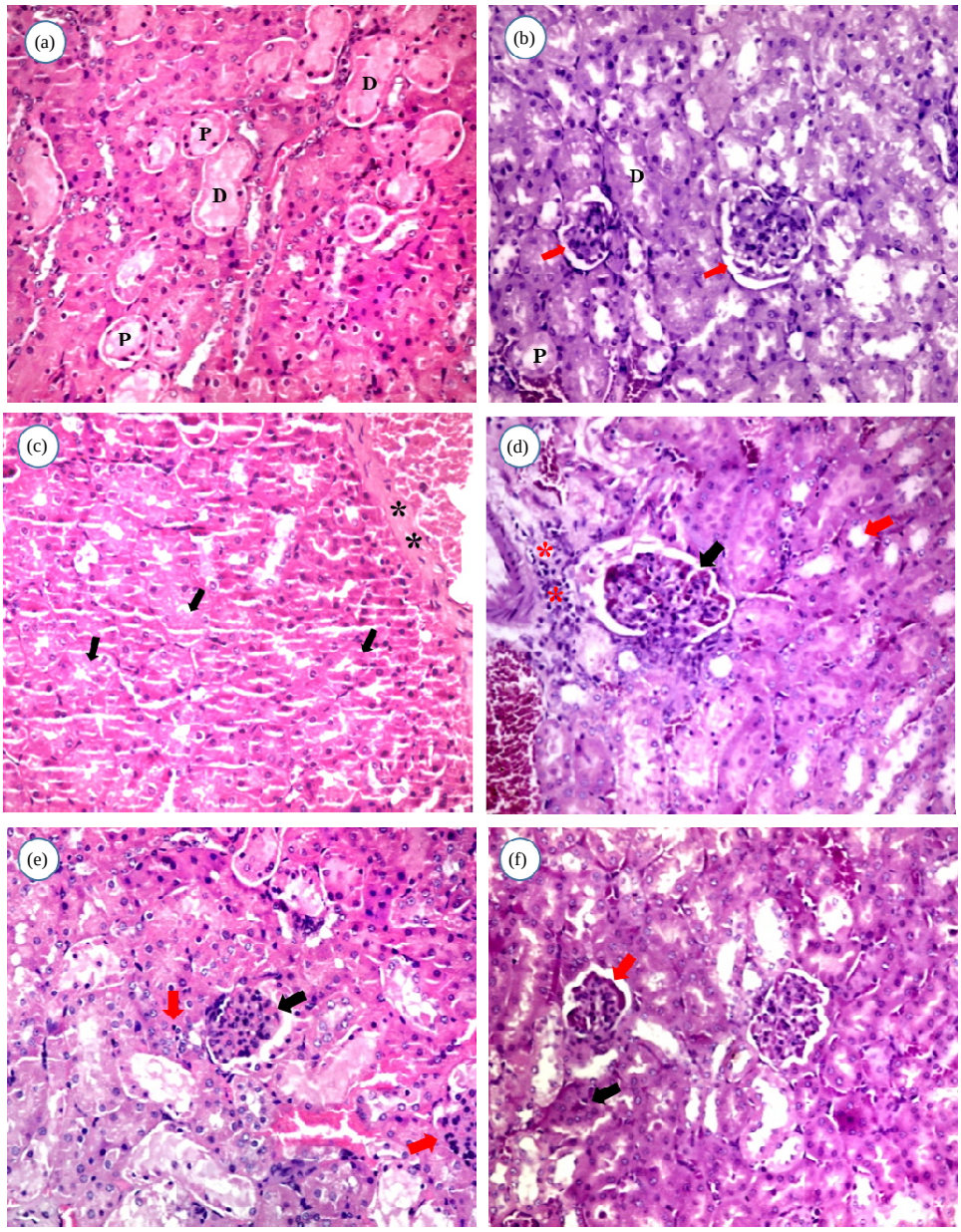


Fig. 7(a-f): Photomicrograph of kidney sections stained with H and E (a) Control groups of rat showing normal proximal (P) and distal (D) renal tubules, (b) Cross section treated with LC showing normal renal cortex formed of normal glomeruli (red arrow) surrounded by normal proximal and distal renal tubules (red arrow), (c) Aspartame low dose ASP-LD treated rats showing hydropic degeneration of the renal tubular epithelial cells (black arrow) and interstitial cells' infiltration (*), (d) ASP-HD showing severe loss of kidney architecture, congested vascular space with enlarged glomeruli (black arrow) and aggregated of inflammatory cells between the renal tubules (*); loss of the brush border of renal tubular epithelium (red arrow), (e) ASP-LD+LC showing normal tubules and glomeruli (black arrow) with few scattered inflammatory cells (red arrow) and (f): ASP-HD+LC treated animals showing severely loss of renal structures with sloughing necrotic cells (black arrow) and markedly atrophic glomeruli (red arrow) (400X)

lumen. The distal convoluted tubules appeared lined with cuboidal epithelium framing a wider lumen (Fig. 7b). ASP-LD treated group showed hydropic degeneration of the renal tubular epithelial cells and some interstitial cells' infiltration (Fig. 7c). ASP-HD treated group showed severe loss of kidney architecture with severe changes in the renal cortex,

Table 3: Effect of aspartame and/or LC on DNA damage of kidney measured as comet percentage tail damage and tail moment

| Groups | Tail length (px) | DNA in tail (%) | Tail moment (μm) |
|---|---|---|---|
| Control | 2.20±0.98 | 0.56±1.02 | 0.96±0.02 |
| LC | 2.15±0.56 | 0.72±1.32 | 0.54±0.14 |
| ASP-LD | 17.16±2.74[a] | 22.15±2.56[a] | 11.75±2.95[a] |
| ASP-HD | 21.81±2.68[a] | 33.73±3.65[a] | 15.63±1.36[a] |
| ASP-LD+LC | 8.28±0.74[b] | 10.41±2.32[b] | 4.86±0.25[b] |
| ASP-HD+LC | 9.07±0.68[b] | 11.61±2.35[b] | 5.35±0.88[b] |

Values are expressed as Mean±SE, n = 8 for each treatment group, LC: L-carnitine, ASP-LD: Low dose of aspartame and ASP-HD: High dose of aspartame, [a]Significant difference as compared to control and [b]Significant difference as compared to ASP-LD ($p \leq 0.05$)

congested vascular space and aggregated of inflammatory cells between the renal tubules with enlarged glomeruli and lack of the brush border of renal tubular epithelium (Fig. 7d). Rats that treated with ASP-LD+LC showed normal tubules and glomeruli with few scattered inflammatory cells (Fig. 7e). High dose of ASP and LC treated animals showing severely loss of renal structure with sloughing necrotic cells and markedly atrophic glomeruli (Fig. 7f).

## DISCUSSION

The current study focused on the biochemical, ultrastructure, histopathological alterations and genotoxicity investigation to prove that LC has an ameliorative effect on ASP induced renal toxicity.

The marked elevation in creatinine and urea would be due to the ASP toxic effect on kidney and induce nephritic damage. These effects diminished glomerular filtration average and reservation of urea and creatinine as recorded by Amin *et al.*[21].

Many pathological alterations prolonged to cellular injury takes place if there is the imbalance in tissues between oxidants and anti-oxidants. Oxidative stress damage is the marker of ASP toxicity. In the present study, MDA and C-reactive protein levels in ASP group were elevated markedly and joined by a remarkable decrement in the activities of antioxidant enzymes (SOD, CAT and GPx) as well as GSH and total protein levels in the kidney tissues in comparison with the control group. The current results were similar with previously reported by Iman[22] study who demonstrated that ASP may elicit rat's liver and kidney oxidative stress. Elevation of LPO levels in ASP-treated rats indicated the free radicals production and had been used as indirect biomarkers of oxidative stress. Antioxidant enzymes such as SOD and CAT that are decreased in ASP treated groups are considered to be primary defense enzymatic antioxidants that prevent the oxidative damage by ROS to macromolecules[23]. Ashok *et al.*[24] found that methanol is produced as a metabolite of ASP exposure after 24 h. Also, other previous investigation found that methanol administration which is a metabolite of ASP could decline the enzymatic antioxidant (SOD, CAT and GPx) in the lymphoid organs[25,26]. Moreover, ASP found to be induced ROS formation and cytotoxicity due to an imbalance in the antioxidant/pro-oxidant status in the hepatic tissues[27-29].

The CAT and GPx protect SOD enzyme against $H_2O_2$ inactivation. Alternately, SOD protects CAT and GPx against superoxide anion through dismutation of endogenous cytotoxic superoxide anion to $O_2$ and $H_2O_2$ [30]. However, overload of free radical could have been disturbing these regulations. Furthermore, the decrease in the activities of SOD and CAT may be due to the formation of formaldehyde from the methanol as the ASP metabolism. This is in correspondence with Mukaddes *et al.*[31] who specified that formaldehyde disclosure leads to a reduction in activities of SOD and CAT in liver tissues.

The GSH levels decreased in ASP-treated groups after 30 days of administration. The reduction in renal GSH level increases oxidative stress susceptibility of the cell. The GSH is considered to be one of the most important non-enzymatic antioxidant in living cells[32].

In the present study, the ASP administration induced essential changes in the structures of the kidney histologically. Moreover, it was noticed that ASP led to histological structural alterations in renal tissue. In the present study, there was dilution in the lumen of the proximal and distal tubules in ASP treated rats. The cortex was affected more than other parts of the kidney; because the renal cortex encountered the most of blood nutrient flow to the organ. Therefore, a high amount of the toxin reached the renal cortex[33]. It was suggested that ASP afforded apoptotic changes in most of the renal tubule padding cells that could be secondary to DNA damage as the effect of ASP metabolites[34]. The cytological changes results are agreed with Butchko *et al.*[35] and El Haliem and Mohamed[36]. who stated that such hepatic fibrosis seemed to be due to direct toxic effects of ASP which induced proliferation of the hepatic fibrous connective tissue.

Although the FAD/WHO reported that the sweeteners are safe to be used in food, the comet assay in the current study showed that ASP is genotoxic in the kidney. The comet assay used as a sensitive method to assess the DNA damage.

Increasing the DNA damage by increasing the percentage of tail DNA was recorded in the ASP-treated group. DNA damage could be due to the generation of free radical which cause DNA strand break down[37].

The comet parameters of DNA were elevated in the bone marrow cells of Swiss Albino mice due to the low-dose range (7-37 mg/b.wt. kg) of ASP that induced DNA strand breaks, as revealed by the increment of the DNA percentage in the tail[38]. However, Al Suhaibani[39] observed that ASP is not genotoxic at low concentration. Administration of ASP orally to the pregnant rats caused cytogenetic effects in the mothers and the offspring[40]. Therefore, the histological alterations could be secondary to DNA injury as mentioned before by Trocho *et al.*[34] who found the chronic use of ASP led to DNA impairment. This damage plays a definitive role in the initiation of apoptosis.

The LC is necessary for the β-oxidation of fatty acids by raise inhibition of glycolysis and amplifies the fatty acid metabolism[41]. Results from the present study suggested that LC administration was advantageous in inhibiting oxidative stress and activating antioxidant enzymes through its role as an antioxidant with protective effects against free radical damage.

The histological monitoring basically reinforced the results obtained from enzyme assays. There was a significant reversion of these enzyme levels after treatment with LC. This is in accordance with the ordinarily accepted view that serum levels of transaminases restore to normal with the regeneration of hepatocytes.

The results proved that using LC along with ASP administration show reinforcement and reduction in oxidative stress parameters in ASP groups. The MDA was significantly decreased while, CAT, GPx and SOD activities as well as total protein and GSH levels were elevated. These effects of LC may result directly from effects of antioxidant against free radicals or from promoted biosynthesis of antioxidants non-enzymatic/enzymatic.

The histological results revealed that treatment of the kidney with LC and ASP showed amelioration in Malpighian corpuscles. Therefore, it is possible that LC could capture free radicals and generate beneficial effects against ASP injury of the kidney.

In the current study, there were changes in the renal corpuscle that could be due to glomerulus shrinkage. The increasing the thickening of the basement membrane of glomerular might be due to an elevation in the glycoproteins deposition as described before by Hotta *et al.*[42] and Hamza *et al.*[43] after treatment the kidney with dimethylnitrosamine .There is still damage in the kidney at the cellular level in rats treated with LC and ASP that might be a result of the excessively production of ROS as well as decreasing the intrinsic antioxidant by ASP in the cells. In addition to this, ASP caused DNA damage.

## CONCLUSION

The ASP caused renal damage and effect on their functions as proved by significant biochemical and histological changes as well as DNA strand breaks. LC supplied protection from these changes and declined the injury afforded by ASP. The treatment of kidney with LC and ASP showed an improvement in renal corpuscles. Therefore, it is possible that LC could scavenge free radicals and produce beneficial effects against ASP damage of kidney. Moreover, the ASP intake should be restricted and administrated with LC when it is used in food or beverages.

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