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# Effect of multi-walled carbon nanotubes on kidney of male albino rats with the potential ameliorative effect of alpha lipoic acid

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**ABSTRACT**

Multi-walled carbon nanotubes (MWCNTs) have been widely used in various industrial and medical applications. Alpha lipoic acid (ALA) plays a great role in the antioxidant defense system. This research was conducted to investigate the potential therapeutic effect of ALA on MWCNTs induced nephrotoxicity in rat. Forty albino rats were assigned into four equal groups: Group I (Control) was treated with 1% tween-80 (0.25 mL/rat/IP) for 5 days, followed by distilled water (2 mL/kg/PO) for 10 days, group II (ALA) was orally administered ALA suspension (200 mg/kg) for 10 days, group III (MWCNTs) was intraperitoneally injected with MWCNTs suspension at the concentration of 0.5 mg/kg daily for 5 days followed by (2 mL/kg per day) distilled water for 10 days, and group IV (MWCNTs+ALA) was treated with MWCNTs (0.5 mg/kg, once, IP) for 5 days followed by ALA (200 mg/kg, PO) for 10 days. At the end of experiment, the rats were euthanized. Blood and kidney samples were collected from all rats for biochemical, kidney for histopathological, and immunohistochemical analyses. MWCNTs substantially increased blood urea nitrogen, creatinine, and malondialdehyde. Meanwhile, they markedly reduced glutathione levels. Additionally, MWCNTs induced several histopathological alterations, including dilatation and congestion of most glomeruli, degenerative changes of renal tubules and prominent interstitial hemorrhage. A significant increase in area percentage of caspase 3 and COX2 in MWCNTs exposed rats. On the other hand, ALA administration alleviated the adverse toxic effects induced by MWCNTs. Conclusion, ALA significantly ameliorate MWCNTs-induced nephrotoxicity through antioxidant, anti-inflammatory, and antiapoptotic mechanisms.

## Introduction

Nanotechnology is a fast-developing industry that has a significant effect on society and environment. Potential health and environmental impacts of nanomaterials must be extensively evaluated before their broad commercialization (Patlolla *et al*., 2011). Carbon nanotubes (CNTs) are one of the most revolutionary (Gazia and El-Magd, 2019) and versatile nanomaterials (Albini *et al*., 2015).

Depending on the number of graphene layers, CNTs are categorized into single walled CNTs and Multi walled CNTs (MWCNTs) (Liu *et al*., 2009). They are broadly used, either as it is or after modification, in commercial environmental and energetic sectors owing to their extraordinary physicochemical properties and distinctive one - dimensional hollow nanostructure (Johnston *et al*., 2010). Moreover, CNTs have a wide medical application including biosensors (Gruner, 2006), delivery of drugs and vaccines (Klumpp *et al*., 2006), gene therapy (Mohseni-Dargah *et al*., 2019), and synthesis of novel biomaterials for tissue engineering of bone (Flores-Cedillo *et al*., 2016). Additionally, MWCNTs are used as coatings and additives in plastics (Albini *et al*., 2015).

Consequently, humans are progressively exposed to CNTs through production, use and disposal (Johnston *et al*., 2013; Winkler *et al*., 2013; Hristozov *et al*., 2014) via multiple routes as inhalation, skin contact, and intravenous injection in medical uses (Liu *et al*., 2014). The high exposure to CNTs makes an extensive concern regarding their potential toxicity. Recently, many in vitro and in vivo studies have shown that MWCNTs can induce adverse health effects. Liver, kidney, heart, and brain are among the organs that are susceptible to MWCNTs accumulation and toxicity (Mercer *et al*., 2013). The most well-established mechanism of MWCNTs toxicity is the physical interaction of CNTs with cellular and extracellular components resulting in alteration of vital cell functions (Maynard *et al*., 2004). Additionally, CNTs induce the production of high level of ROS which subsequently lead to detrimental impacts on the cell represented by apoptosis, damage of genetic material, amino acids' oxidation, and inactivation of enzymes (Florek *et al*., 2023).

The kidney is especially vulnerable to toxins owing to its high blood supply for blood filtration and the elimination of toxic substances and their metabolites from the body (Pizzorno, 2015; Gazia and El-Magd, 2019; Florek *et al*., 2023). In the same manner, Pujalté *et al*. (2011) illustrated that CNTs, in the systemic circulation, can be excreted by renal clearance. Thus, it is thought that kidney is one of the most susceptible organs to the harmful effect of carbon nanoparticles (Gazia and El-Magd, 2019). MWCNTs deposition (Mercer *et al*., 2013; Albini *et al*., 2015) increasing the ROS production (Zamani *et al*., 2021) that leads to peroxidation of lipid, protein, and DNA (Sarhan and Hussein, 2014), reducing the level of GSH (Florek *et al*., 2023) and development of marked histopathological alterations (Gazia and El-Magd, 2019).

Alpha lipoic acid (ALA) or thioctic acid [1,2-dithiolane-3-pentanoic acid] is a naturally occurring disulfide compound, synthesized by an enzymatic pathway in plants and animals' mitochondria from cysteine and octanoic acid as sulphur sources (Quan *et al*., 2001). It is recognized as a powerful antioxidant owing to its ability to prevent peroxidation of lipid (Turkyilmaz *et al*., 2020), quench the reactive oxygen species (ROS) like hydroxyl radicals, superoxide anion radicals, singlet oxygen, peroxyl radicals, hypochloric acid and hydrogen peroxide (Feng *et al*., 2013; Oktan *et al*., 2021). Additionally, ALA has the ability to regenerate other natural antioxidants like vitamin E or vitamin C from their radical or inert forms (Malarkodi al., 2004; Feng *et al*., 2013) and promote the production of other antioxidants, such as glutathione (Bilska *et al*., 2007). Besides, it has


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anti-inflammatory and anti-apoptotic properties (Suh *et al*., 2015). It was reported that ALA has a favourable effect on drug-induced nephrotoxicity in the experimental models (Somani *et al*., 2000; Murugavel and Pari, 2004; Kang *et al*., 2009; Lee *et al*., 2009; ÇAKIR *et al*., 2015; Cavdar *et al*., 2020). Moreover, several investigations have been reported that administration of ALA is beneficial in the prevention or treatment of diabetes, polyneuropathy, cataract, and neurodegeneration (Takaoka *et al*., 2002; Amudha *et al*., 2007).

To the best of our knowledge, this is the first study which clarified the potential alleviative effect of ALA against MWCNTs induced renal damage. The therapeutic effect of ALA was evaluated by measuring levels of renal function parameters and oxidative stress biomarkers, as well as by histopathological and immunohistochemical investigation of rats' kidneys in all groups.

## Materials and methods

*Chemicals*

Alpha Lipoic Acid (100 mg capsule, Puritans' Pride, Inc., USA) was purchased from Health Shop, Egypt. Multi-walled carbon nanotubes (MWCNTs) were manufactured by Nano Gate company (Cairo, Egypt) using catalytic chemical vapor deposition technique (Patlolla *et al*., 2011). Tween 80 was obtained from Sigma - Aldrich.

*Experimental protocol and animal grouping*

Experimental Animals and Ethical Approval

Forty male albino rats (6-8 weeks old, 100-130 g) were purchased from the animal house of Vaccera, Helwan, Egypt. Animals were kept in the animal unit facility, Faculty of Veterinary Medicine, Cairo University. The rats were maintained in polypropylene cages (43x40x29 cm) with five rats per cage with soft wood shavings employed as bedding. Before the experiment, animals were housed for two weeks to acclimatize at a temperature of 22.0±2.0°C with constant humidity (50-70%), and 12:12 h light: dark cycles. Animals were fed a commercially balanced diet and water ad libitum. All experimental procedures were performed in compliance with the National Institutes of Health (NIH) guidelines and were approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University (approval no: Vet CU 08072023727).

Experimental Design

After acclimatization period, animals were randomly allocated to 4 equivalent groups (n = 10 rats/ group). Group I (Control group), animals were treated via intraperitoneal (IP) route with 1% tween-80 (0.25 mL/ rat) for 5 consecutive days, followed by 2 mL/kg per day distilled water via oral gavage for 10 successive days. Group II (ALA group), rats were orally administered ALA suspended in distilled water (1 mL/rat) at the concentration of 200 mg/kg daily for 10 days (Memudu and Adewumi, 2021) followed by 2 mL/kg per day distilled water via oral gavage for 5 successive days. Group III (MWCNTs group), rats were intraperitoneally injected with MWCNTs suspended in 1% tween-80 (0.25 mL/rat) at the concentration of 0.5 mg/kg daily for 5 consecutive days (Adedara *et al*., 2018) followed by 2 mL/kg per day distilled water for 10 successive days. Group IV, rats were treated with MWCNTs (0.5 mg/kg, once, IP) for 5 consecutive days, followed by ALA (200 mg/kg, PO) daily for 10 days.

*Sample collection and preparation*

At the end of the experiment, all animals were anaesthetized with diethyl ether so that blood samples could be obtained from the ocular plexus of all rats in clean glass tubes, and then allowed to clot for 20 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate serum that were stored at -20°C for determination of kidney function tests. Afterword, rats were sacrificed by cervical dislocation, and kidneys were immediately removed from each rat. Samples for the assessment of oxidative stress parameters were stored at -80°C. Other samples were histopathologically and immunohistochemically examined after being preserved in 10 % neutral buffered formalin for 24 - 48 h.

*Biochemical Analyses*

Renal function tests

Blood urea nitrogen (BUN) was assayed using urease colorimetric method and serum level of creatinine was determined by Kinetic jaffé reaction. The procedures were conducted according to reagent kits following the given instructions (spectrum diagnostics. Egyptian Company for Biotechnology).

Renal oxidative stress biomarkers

Tissue of kidney was homogenized in an ice-cold 0.1-M phosphate-buffered saline (pH 7.4) using tissue homogenizer. The crude tissue homogenate was centrifuged at 15,000 rpm for 15 min at 4°C and used to determine malondialdehyde (MDA) according to Ohkawa *et al*. (1979), reduced glutathione (GSH) according to Ellman (1959).

*Histopathology and immunohistochemistry*

Light microscopy

Fixed kidney specimens were dehydrated in ascending dilutions of ethanol. After that, the samples were cleared in two changes of xylene, embedded in paraffin wax, and then cut to obtain 3-4 µm paraffin sections. De-waxed serial sections were stained with hematoxylin and eosin (H&E) for histopathological investigation (Bancroft and Gamble, 2013).

*Immunohistochemistry*

Caspase - 3

Selected kidney sections were immunohistochemically stained to clarify the expression of caspase-3 [inactive caspase-3 (CPP32) Ab-4, rabbit polyclonal antibody, Neomarker, Fremont, CA, USA at dilution 1/100 via avidin-biotin peroxidase complex method (Ramos-Vara, 2005).

Cyclooxygenase Enzyme 2 (COX-2)

Different kidney sections were deparaffinized in xylene and rehydrated in graded alcohol. Slides were immersed in hydrogen peroxide for deactivation of the endogenous peroxidase. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) for 10 min in the microwave oven at 500 W. After incubation with blocking serum (Ultra-V blocking solution, Thermo Scientific, USA) for five minutes, the slides were incubated overnight at 4ºC in a humidified chamber with the primary antisera to COX 2 (Rabbit anti-COX2 polyclonal antibody, Cayman Chemical, Ann Harbor, MI at a dilution of 1:50). The universally biotinylated goat anti-rabbit antibody (Thermo Scientific, USA) was added and incubated for 10 minutes. Finally, sections were incubated with streptavidin peroxidase (Thermo scientific, USA). The peroxidase activity was visualized with 3, 3 diaminobenzidine tetrahydrochloride (DAB, Sigma) producing a brown color. The slides were washed with phosphate buffer saline after each step. After that, the slides were counterstained with haematoxylin then dehydrated and mounted. Primary antibody was omitted and replaced by

PBS for negative (Ramos-Vara, 2005).

Evaluation of immunohistochemical observations (Area percent)

Both caspase 3 and COX-2-stained renal sections were evaluated using Leica Quin 500 analyzer computer system (Leica Microsystems, Switzerland) in the Faculty of Dentistry, Cairo University. The image analyzer was calibrated automatically to change pixels into actual micrometer units. Caspase 3 and COX-2 immunoreaction were measured as percent of total area in a standard measuring frame in 10 fields from different slides in each group using magnification (X400) by light microscopy. All areas demonstrating caspase 3 and COX-2 positive brown immunostaining were selected for assessment regardless the strength of the immunostaining. Mean value and standard error of the mean (SEM) were obtained for each specimen and statistically analyzed.

*Statistical Analysis*

All quantitative findings were analyzed by SPSS version 17.0 software (IBM, USA). Data were expressed as mean±SEM and statistically analyzed using one-way analysis of variance (ANOVA) followed by LSD post hoc test. P-value ≤ 0.05 was considered statistically significant.

## Results

*Biochemical investigation*

Effect of MWCNTs and ALA on kidney function

Renal damage was evaluated by using BUN and creatinine levels. Fig. 1 reveals that MWCNTs induced a significant increase in BUN level from 4.85 to 6.3 mg/dL and serum creatinine level from 0.54 to 0.80 mg/dL when compared with the control group. In comparison with MWCNTs group, treatment with ALA significantly reduced BUN. Similarly, treatment with ALA significantly reduced serum creatinine.

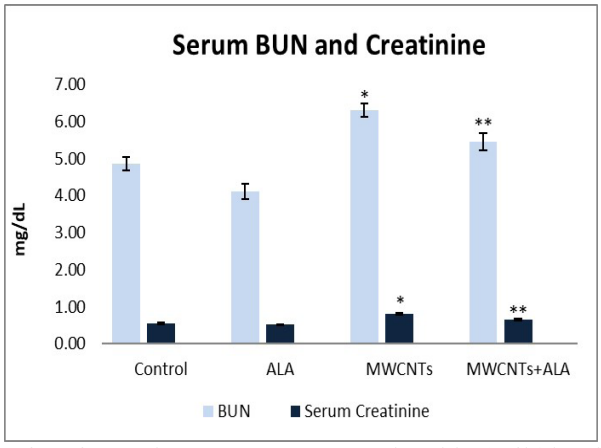


Fig.1. Effects of ALA and MWCNTs on serum BUN and creatinine (mg/dL) in male rat. Data are represented as mean ± SEM. *indicates significant difference from the corresponding control negative group at p ≤ 0.05. ** indicates significant difference from the MWCNTs treated group at p ≤ 0.05.

Effect of MWCNTs and ALA on oxidative stress biomarkers

*Renal content of GSH*

Compared with the control group, renal GSH content was significantly reduced from 489.5 to 370.43 µM $g^{-1}$ in rats exposed to MWCNTs. Treatment with ALA significantly elevated GSH in comparison with MWCNTs treated group as shown in Fig. 2.

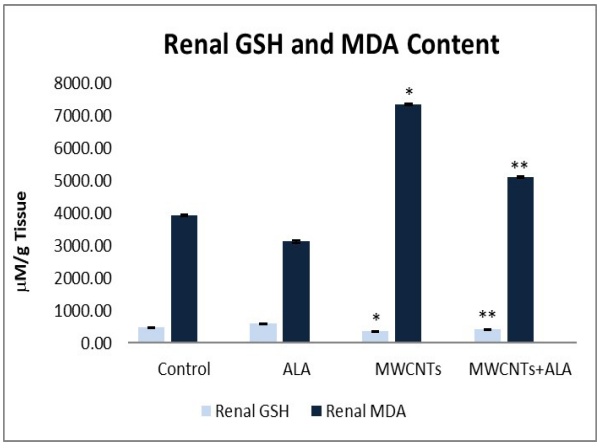


Fig. 2. Effects of ALA and MWCNTs on renal GSH and MDA (µM g-1 tissue) in male rat. Data are represented as mean ± SEM. *indicates significant difference from the corresponding control negative group at p ≤ 0.05. ** indicates significant difference from the MWCNTs treated group at p ≤ 0.05.

*MDA Content in renal tissue*

According to the data obtained in Fig. 2, treatment with MWCNTs significantly increased renal MDA content from 3928.76 to 7344 µM g-1 tissue when compared with the control group. On the other hand, ALA treatment successed to induce a significant reduction in renal MDA content to 5098.67 µM $g^{-1}$ tissue in comparison with MWCNTs group.

*Histopathological examination*

Light-microscopy findings

H&E-stained kidney sections obtained from the control group (GP I) and ALA administered group (GP II) showed a normal histological structure of renal cortex that consisted of renal corpuscle, proximal and distal convoluted tubules. Renal corpuscle composed of a glomerular capillary tuft surrounded by the double walled (parietal and visceral layers) Bowman's capsule separated by a patent urinary space. Proximal convoluted tubules (PCT) were lined by truncated pyramidal cells with narrow lumen. Distal convoluted tubules (DCT) were lined by cuboidal cells with broad lumina (Fig. 3a and Fig. 3b) respectively.

On the other hand, renal tissue sections obtained from male albino rats in experimental group exposed to MWCNTs (GP III) displayed several histopathological alterations in the renal cortex compared with the control group. Most renal corpuscles showed dilatation and congestion of the glomerular capillaries (Fig. 3c) while, others exhibited degeneration and shrinkage of glomeruli with a wide capsular space (Fig. 3d). Most PCT and DCT displayed disorganized architecture with shedding of the cellular cytoplasm into the tubular lumina. Some cells of PCT appeared with flattened pyknotic nuclei and others displayed loss of their nuclei into the tubular lumen. Additionally, some degenerated DCT appeared with desquamated epithelial cells and others demonstrated partial loss of cytoplasmic acidophilia of some tubular cells. Also, some DCT exhibited loss of the normal cellular architecture with pyknosis of their nuclei which frequently become elongated (Fig. 3c). Vascular congestion and inflammatory cell infiltration (Fig. 3e) in addition, area of hyalinization (Fig. 3d) was noticed in the cortex. However, the MWCNTs-exposed rats treated with ALA (GP IV) showed a marked improvement in the renal histological architecture. The renal cortex appeared nearly normal except for presence of few degenerated tubules with desquamated cells (Fig. 3f).

Furthermore, the sections of renal medulla obtained from the control rats (GP I) and ALA administered rats (GP II) revealed an ordinary histoarchitecture of collecting tubules that were lined by simple cuboidal cells, loop of Henel's and interstitial blood capillaries (Fig. 4a and Fig. 4b). On contrary, the renal tissue of MWCNTs exposed rats (GP III) revealed several histopathological changes of the renal medulla. Most collecting tubules of MWCNTs exposed group showed hydropic degeneration as the

tubular cells appeared swollen with vacuolated cytoplasm and pyknotic nuclei (Fig. 4c), whereas other tubules demonstrated marked attenuation and loss of normal cellular architecture with pyknosis of most tubular cells' nuclei that occasionally appeared flattened. Furthermore, marked interstitial haemorrhage was observed between the degenerated tubules (Fig. 4d). On the other hand, remarkable recovery of the renal medulla with few degenerated collecting tubules as well as slight interstitial haemorrhage were noticed in the MWCNTs exposed group treated with ALA (GP IV) (Fig. 4e).

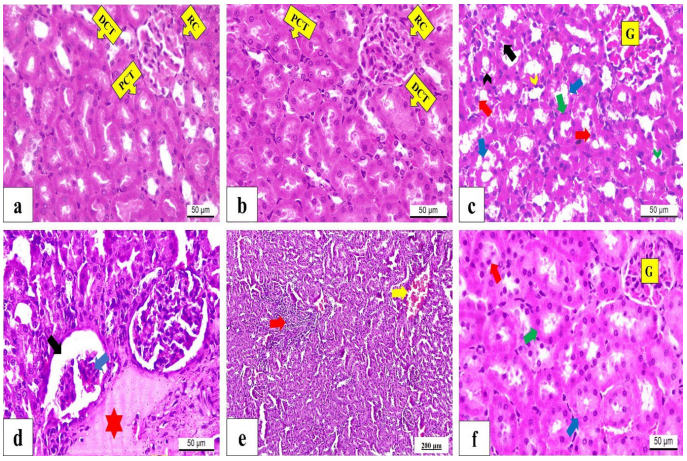


Fig. 3. A photomicrograph of H&E-stained sections of albino rats' renal cortex (a) Control group (GP I) and (b) ALA administered group (GP II) showing normal histological structure of the renal corpuscle (RC), proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) (x400). (c:e) MWCNTs-exposed group (GP III) showing (c) Dilated and congested glomerular capillaries (G), some PCT with flattened pyknotic nuclei of some cells (green arrow) and shedding of cellular cytoplasm (black chevron) and / or some nuclei (green chevron) into the tubular lumina, some DCT showing desquamation of some epithelial cells (red arrows) in addition to, shedding of cytoplasmic content (blue arrows) into the lumen and others demonstrated partial loss of cytoplasmic acidophilia of some lining cells (black arrow). Moreover, degenerated DCT with loss of normal cellular architecture and elongated pyknotic nuclei was observed (yellow chevron). (d) Shrunken and degenerated glomerulus (blue arrow) with a wide urinary space (black arrow). In addition to, area of hyalinization (red star) (x400). (e) Vascular congestion (yellow arrow) and inflammatory cell infiltration in between the tubules (red arrow) (x100). (f) MWCNTs-exposed rats treated with ALA (GP IV) revealing restortion of histological structure of the renal corpuscle with normal glomerulus (G), PCT (blue arrow) and DCT (green arrow) whereas, some tubules still showed degeneration with few desquamated cells (red arrow) (x400).

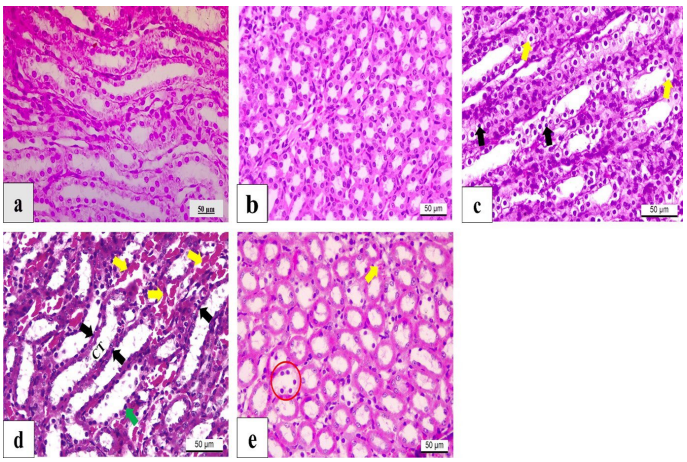


Fig. 4. A photomicrograph of H&E-stained sections of albino rats' renal medulla (a) Control group (GP I) and (b) ALA administered group (GP II) showing intact histoarchitecture of the collecting tubules (x400). (c: d) MWCNTs-exposed group (GP III) showing (c) hydropic degeneration (yellow arrows) was prominent in most collecting tubules with pyknotic nuclei (black arrows), (d) Some collecting tubules (CT) lost their normal histoarchitecture with pyknosis of most nuclei (green arrows) which frequently appeared flattened (black arrows). In addition to, marked interstitial hemorrhage (yellow arrows) (x400). (e) MWCNTs-exposed rats treated with ALA (GP IV) exhibiting remarkable recovery of the renal medulla with few degenerated collecting tubules (red circle) in addition to, slight interstitial hemorrhage (yellow arrow) (x400).

*Immunohistochemical observations*

Immunohistochemistry for caspase- 3

Kidney samples obtained from the control rats (GP I) (Fig. 5a) and ALA exposed rats (GP II) (Fig. 5b) exhibited negligible reaction to caspase - 3. However, strong caspase- 3 immunoexpression was noticed in the renal tubular epithelium of MWCNTs-exposed one (GP III) (Fig. 5c). Meanwhile, the renal sections obtained from the group exposed to MWCNTs and treated with ALA (GP IV) showed a mild immunoreactivity to caspase-3 in few renal tubular epithelium (Fig. 5d).

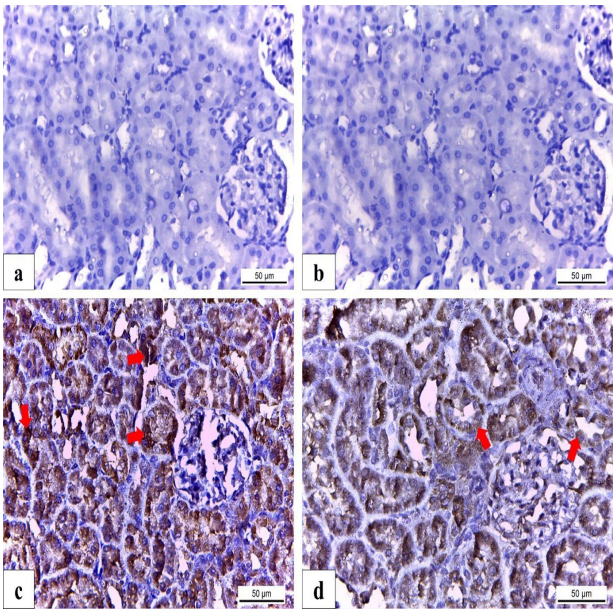


Fig. 5.A photomicrograph of caspase-3 immunoexpression in the renal cortex sections (x400). a&b: Negligible caspase-3 immunoexpression in the renal tissue obtained from the control group (GP I) (a) and ALA exposed group (GP II) (b). (c) Strong positive immunoexpression (red arrows) in the tubular epithelium of MWCNTs-exposed group (GP III). (d) Mild caspase-3 expression (red arrows) in the MWCNTs -exposed group treated with ALA (GP IV).

Immunohistochemistry for COX-2

Renal tissues obtained from both control and ALA exposed groups exhibited negative COX-2 immunoreactivity (Fig. 6a and Fig. 6b) respectively. Whereas MWCNTs-exposed group revealed strong positive immunoreaction for COX-2 in the renal tubular epithelium (Fig. 6c). In contrast, A slight immunostaining for COX-2 was noticed in the group exposed to MWCNTs and treated with ALA (Fig. 6d).

Analysis of the immunohistochemical observations

Data analysis displayed a significant elevation ($p \leq 0.05$) in the area percentage occupied by caspase 3 and Cox-2 positive immunoreactive cells within the renal tissue of MWCNTs exposed group as compared to control group. Conversely, treatment with ALA substantially ($p \leq 0.05$) decreased caspase 3 and Cox-2 area % induced by MWCNTs (Table 1).

Table 1. The ameliorative effect of ALA on the area covered by caspase-3 and Cox-2 positive immune reactive cells in renal tissue of MWCNTs exposed rats.

| Experimental groups | Caspase-3 Area percentage | Cox-2 Area percentage |
|---|---|---|
| Group I | $0.1\pm0.0^{c}$ | $0.0\pm0.0^{b}$ |
| Group II | $0.1\pm0.0^{c}$ | $0.0\pm0.0^{b}$ |
| Group III | $12.8\pm1.3^{a}$ | $15.9\pm1.4^{a}$ |
| Group IV | $6.0\pm1.2^{b}$ | $3.3\pm1.0^{b}$ |

Values are presented as mean±SEM. [a, b, c] The different superscript letters within the same column indicate significant differences with $p \leq 0.05$.

## Discussion

The kidney is one of the most target organs in MWCNTs toxicity (Reyes *et al.*, 2022), but there are some other organs like liver, heart, lung, and brain that were investigated for MWCNTs toxicity (Mercer *et al.*, 2013). This research has been focused on evaluating the ability of oral administration of ALA to attenuate MWCNTs - induced kidney dysfunction.

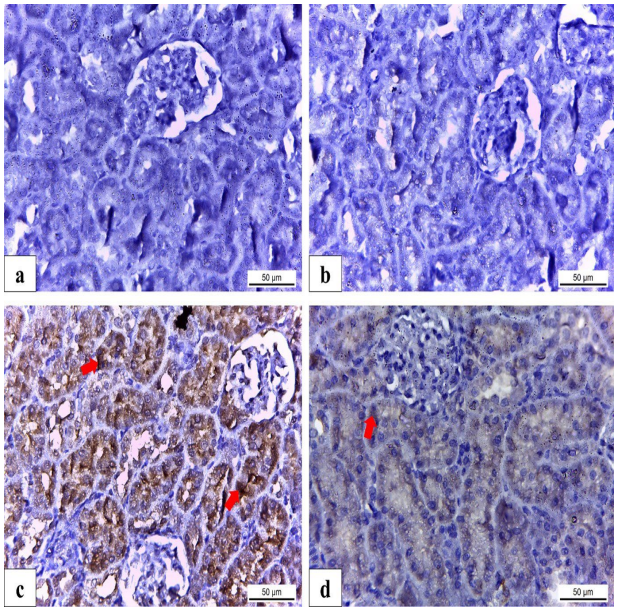


Fig. 6. A photomicrograph revealing COX-2 immunoexpression in the renal cortex sections (x400). a. Control group: b. ALA administered group exhibiting negative COX-2 immunoreaction. c. MWCNTs-exposed group displaying strong COX-2 immunoexpression in the renal tubular epithelium (red arrows). d. MWCNTs-exposed group treated with ALA showing weak immunostaining for COX-2 in few renal tubules (red arrow).

The therapeutic effect of ALA against MWCNTs induced-renal oxidative damage was monitored by assessment of renal biomarkers (BUN and creatinine) and oxidative stress biomarkers (GSH and MDA). BUN is considered the main intense renal biomarker that increases when any sort of renal injury happened. Serum creatinine is another renal biomarker which is elevated in response to hindered glomerular filtration rate (Ahmed *et al*., 2022). Our current results indicated that rat's exposure to MWCNTs elicited renal damage as evidenced by significant increase in serum renal biomarkers, BUN and creatinine, and disruption of the normal renal architecture. These results support the earlier studies which conducted by Awogbindin *et al*. (2021) and Florek *et al*. (2023). MWCNTs induced- renal damage could be attributed to induction of oxidative stress. In the present study, renal oxidative status was monitored by assessment of GSH and MDA. MWCNTs exposure induced oxidative stress i.e., significant decrease in GSH and increase in MDA. GSH is a tripeptide molecule that found in mammalian cells where it serves as a potent reducing agent which neutralize the damaging effects of ROS (Franco *et al*., 2007). Accumulation of reactive oxygen species (ROS) induces the lipid peroxidation of the cellular membrane and production of lipid peroxidative products as MDA (Su *et al*., 2019). In addition to our results, several in vitro and in vivo studies reported the oxidative damage induced by MWCNTs (Shvedova *et al*., 2003; Ye *et al*., 2009; Awogbindin *et al*., 2021; Florek *et al*., 2023).

ALA is a universal antioxidant because it can act as an antioxidant in both hydrophilic and lipophilic conditions to reduce ROS and reactive nitrogen species (RNS) (Khalifa *et al*., 2020). Our results showed that ALA treatment was able to restore renal health as a result of marked suppression in the serum levels of urea and creatinine in addition to improvement of oxidative stress biomarkers. Kamt *et al*. (2023) stated that the renal-protective effect of ALA is due to its antioxidant properties. The antioxidant properties of ALA are associated with its ability to chelate metals such as iron, copper and zinc and regenerate both exogenous and endogenous antioxidants (Tong *et al*., 2022).

In the present study, the renal tissue of MWCNTs-exposed rats (GP III) displayed multiple histopathological alterations as compared with control rats, like dilatation and congestion of the glomerular capillaries, that concides with the results of Meng *et al*. (2011).This dilatation might be due to the direct effect of nanoparticles on the endothelial cells of the blood vessels, which might cause the release of the endothelial relaxation factor nitric oxide and vasodilatation (Xia *et al*., 2006; Gazia and El-Magd, 2019). Moreover, some renal corpuscles displayed shrinkage and degeneration. This finding correlated to Gazia and El-Magd (2019) study.

Some renal tubules showing many alterations including disorganization, loss of normal architecture and shedding of the cytoplasmic contents as strands in the tubular lumen, and some DCT exhibited desquamation of few tubular cells. The same finding was recorded by Gazia and El-Magd (2019) who suggested that cell detachment may be mediated by ROS which disrupt cytoskeletal proteins and deteriorate intercellular adhesion leading to separation and sloughing of the lining cells into the tubular lumen.

In addition, inflammatory cell infiltration was noticed in the renal cortex. In this respect, Boyles *et al*. (2014) illustrated that the exposure to CNTs triggers rapid influx of inflammatory cells as neutrophils, macrophages, and frequently eosinophils into the affected renal tissue which reflects the ability of CNTs to induce inflammatory response in the kidney (Mitchell *et al*., 2007; Yang *et al*., 2008; Gazia and El-Magd, 2019).

Regarding the renal medulla of MWCNTs- exposed rats, marked hydropic degeneration was noticed in the cells of most collecting tubules which might be due to cellular membrane dysfunction, caused by CNTs, that in turn results in massive influx of sodium and water into the cell leading to vacuolar degeneration (Gupta *et al*., 2015).

Additionally, interstitial haemorrhage was seen in the renal medulla that might be attributed to the reduction in the vascular resistance caused by CNTs or as a result of inflammatory response, or tissue hypoxia resulting in increased capillary permeability (Nabeshi *et al*., 2012; Gazia and El-Magd, 2019).

On the other hand, this study revealed that treatment with ALA substantially restored the MWCNTs induced histopathological alterations in the renal tissue reflecting the ameliorative effect of ALA against MWCNTs toxicity. This finding was consistent with that of Şehirli *et al*. (2008); Al-Attar (2010); Abdou and Abdel-Daim (2014) and Oktan *et al*. (2021). The alleviative effect of ALA might be attributed to its potent role in quenching free radicals (Manda *et al*., 2007) as it contains nucleophile that reacts with endogenous electrophiles including reactive drug metabolites, free radicals, and heavy metals (Biewenga *et al*., 1997). Furthermore, a substantial amount of ALA is converted to dihydrolipoate (DHLA) in the cell (Rochette *et al*., 2013) via lipoamide dehydrogenase, glutathione reductase, and thioredoxin reductase in the presence of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) (Mohamed and Meligy, 2018). Upon the release of DHLA into the extracellular milieu, it reduces cystine to cysteine and thus promotes the production of glutathione (Sen, 1998) that is considered one of the most abundant antioxidants protecting the cellular structure from oxidative damage (Hsu *et al*., 2002).

This investigation exhibited that the renal tissue of MWCNTs exposed rats showed a significant increase in the immunoreactivity of caspase 3 as compared to the control group. This result was in agreement with Gazia and El-Magd (2019) finding. The release of caspase 3 can be induced either by extrinsic or intrinsic factors mediating mitochondrial stress and it plays a vital role in cell apoptosis (Elkeiy *et al*., 2020; Jiang *et al*., 2020). It is well known that mitochondria are the major cell organelles which have been altered by nanoparticle toxicity (Unfried *et al*., 2007; Elkeiy *et al*., 2020). In this respect Tokunaga *et al*. (2003); Barillet *et al*. (2010) and Reddy *et al*. (2010) suggested that apoptosis mediated by carbon nanoparticles (CNPs) might be attributed to the massive release of ROS which cause impairment of the mitochondrial function in the renal tubular cells in vitro.

In contrast, the renal tubular epithelium of MWCNTs exposed rats treated with ALA showed a pronounced reduction in caspase 3 immunoreactivity in this study. Hence, the effective antiapoptotic action of ALA has been commonly assumed as one of the crucial mechanisms. This activity was formerly mentioned (El-Beshbishy *et al*., 2011; Cavdar *et al*., 2021; Oktan *et al*., 2021). Several investigations have shown the potential alleviative effect of ALA against oxidative stress and cell death (Oktan *et al*., 2021; Zhang *et al*., 2023) which might be attributed to the ability of ALA to improve antioxidant defense system and quench free radicals effectively (El-Mancy *et al*., 2022), as well as inhibits mitochondrial apoptotic pathway (Wei *et al*., 2015).

Cyclooxygenase (COX) is an essential enzyme in regulating synthesis of prostaglandins. COX 2 is undetectable in several normal organs and is an inducible isoenzyme in several tissues subjected to inflammation (Simmons *et al*., 2004; Ricciotti and FitzGerald, 2011). In this study, the immunohistochemical examination showed intense COX 2 immunoreaction in the renal tubular epithelium of rats exposed to MWCNTs. This result suggested that MWCNTs could induce inflammation which is in consistent with the study of Gazia and El-Magd (2019) who reported that CNTs can initiate inflammatory response through oxidative stress which promotes the release of cytokines leading to a significant infiltration of tubular leucocytes resulting in an inflammatory environment and finally kidney damage. On the other hand, this research showed that the immunoexpression of COX 2 was significantly reduced in MWCNTs exposed rats administered ALA. This indicates the effective anti-inflammatory role of ALA as it down regulates nuclear factor kappa B (NF-κB) signaling which in turn affect the expression of many other inflammatory genes, such as interleukin-1(IL-1) and interleukin-6 (IL-6), a tissue factor, and tumor necrosis factor alpha (TNF-α) in numerous cell types (Zhang and Frei, 2001; Cho *et al*., 2004).

## Conclusion

The use of MWCNTs has been extremely increased owing to their

physicochemical properties and distinctive tubular morphology. Humans are progressively exposed to MWCNTs, so it makes a great public health concern regarding their potential toxicity. This toxicity was noticed through a substantial increase in BUN, creatinine, and MDA levels besides a reduction in the GSH concentration. Moreover, marked alteration and distortion of the renal tissue as well as strong positive immunoreaction of caspase 3 and Cox 2 were noticed in the MWCNTs - exposed group. These findings reflect that oxidative stress is a main risk factor in MWCNTs induced nephrotoxicity. Interestingly, oral administration of ALA (200 mg/kg/day) for 10 days can substantially enhance the renal function parameters, mitigate oxidative damage, histopathological alterations, inflammation, and apoptosis exerted by MWCNTs intoxication (0.5 mg/kg/IP daily) for 5 days. These observations indicate that ALA improve the antioxidant defense system in addition to its antiapoptotic and anti-inflammatory activities. Collectively, this study verified for the first time that ALA had a potent therapeutic effect against MWCNTs - induced renal toxicity.

## Conflict of interest

The authors declare that they have no conflict of interest.

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