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*Full Length Research Paper*

# Effect of vitamin E and α-lipoic acid on nano zinc oxide induced renal cytotoxicity in Rats

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**The objective of this study is to detect toxic effects of two different doses of nano zinc oxide (ZnO-NP) particles. Moreover, a comparative study was conducted to modulate this toxic effect by the use of two natural antioxidants vitamin E (Vit E) and α-lipoic acid (α-Lip). The results of the current study revealed that ZnO-NP treatment produced hazard effects which were confirmed by the elevation of vascular endothelium growth factor (VEGF) as well as nitric oxide levels in rat serum. Inflammatory markers, including tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6), C-reactive protein (CRP) and IgG were also elevated in rat serum compared to control normal group. Additionally, blood glucose level, as well as serum urea, and creatinine levels were significantly increased in rats intoxicated with ZnO-NP compared to normal control group. On the other hand, reduced glutathione (GSH) level was decreased in renal tissue. These biochemical findings were supported by the histopathological examination of renal tissue and the hazardous effects were dose dependant. Treatment of rats with Vit E or α–Lip along with ZnO-NP administration significantly alleviates most of the elevated previous biochemical parameters. Histopathological examination revealed that animals that received α-Lip or Vit E along with ZnO-NP showed moderate histopathological changes in the form of shrinkage and fragmentation of moderate number of glomeruli with exfoliation of tubular epithelial cells and tubular casts in moderate number of renal tubules. It was concluded that treatment with either α-Lip or Vit E has a beneficial effect against ZnO-NP oxidative stress and related vascular complications.**

**Key words:** C-reactive protein (CRP), interleukin-6 (IL-6), nano zinc oxide, tumor necrosis factor alpha (TNF-α), vascular endothelium growth factor (VEGF).

## INTRODUCTION

Nanoparticles are potential hazardous compound that can stick to cell membrane and penetrate into specific cells in the body. The surface of the nanoparticles could be modified and adapted to the environmental change so as to avoid the recognition and elimination by the human body (Salata, 2004). Nanoparticles could translocate from the lumen of the intestinal tract via aggregations of intestinal lymphatic tissue (Peyer's patches [PP]) containing M-cells (specialized phagocytic enterocytes) (Delie, 1998). Accidental or involuntary contact during production or use is most likely to happen via the lungs from where a rapid translocation through the blood stream is possible to other vital organs (Brook et al., 2004). Due to their small size, nanoparticles can translocate from these entry portals into the circulatory and lymphatic systems, and ultimately to body tissues and organs. Some nanoparticles, depending on their

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composition and size, can produce irreversible damage to cells by oxidative stress or/and organelle injury (Nam et al., 2003).

The distribution of these NP to other organs, such as liver, spleen, brain, heart and kidney may lead to dysfunction of these organs as well. Exposure to some nanoparticles is associated to the occurrence of autoimmune diseases such as systemic lupus erythematous, scleroderma a, and rheumatoid arthritis (Buzea et al., 2007). It has also been proposed that the size of NP surface area greatly increases their ability to produce reactive oxygen species (ROS) (Moller et al., 2010). Published data indicated that numerous metallic elements are selective nephrotoxins that preferentially accumulate and produce cellular injury in the kidney which permits unrestricted use, distribution and reproduction in any medium (Igor Pujalté et al., 2011).

Alteration in total reduced glutathione (GSH) (tGSH) level content in cells can be considered as an indication of adaptive response of the cell to oxidative damage. Nanoparticles of ZnO at high concentrations significantly decreased the tGSH level compared with control values, indicating functional damage to kidney tissues (Moron, 1979). GSH is essential for maintaining cellular integrity. Thus, it protects cells from oxidative damage. GSH also has a major role in restoring other free radical scavengers and antioxidants such as Vit E and L-ascorbic acid (Ankush Gupta et al., 2011). GSH is able to conjugate with endogenous or exogenous substances and prepare them to eventual excretion; this occurs enzymatically by series of enzymes called glutathione transferase. This detoxification of compounds by conjugation with GSH occurs mainly in kidney and liver (Lomaestro and Malone, 1995). Tumor necrosis factor alpha (TNF-α) is an important upstream regulator of various cytokines induced in response to diverse stimuli as well as ROS. Recently, *in vivo* cell experiments showed that exposure to ZnO-NP resulted in oxidative damage and inflammation response in vascular endothelial cells (Gojova et al., 2007). Considering the hazards of treatment failure, drug resistance and heavy costs associated with current drug therapy, natural products and medicinal plants have attracted interest of many researchers in this field (Innsan et al., 2011; Gavanji et al., 2011; Lin et al., 2011).

α–Lipoic acid is a natural cofactor for pyruvate dehydrogenase complex that occurs in the mitochondria of different tissues as liver, kidney and heart tissues (Biewenga et al., 1997). α-Lipoic (α–Lip) acid is a potent antioxidant. Three distinct antioxidant actions of α-Lip and its reduced form, dihydrolipoic acid, have been observed to posses reactive oxygen species scavenging activity; capacity to regenerate endogenous antioxidants such as glutathione and vitamins C and E and metal-chelating activity (Biewenga et al., 1997; Packer et al., 1995). A-Lipoic acid administration to obese Zucker rats improves insulin-stimulated glucose uptake in muscle (Jacob et al., 1996; Streeper et al., 1997). α–Lipoic acid was reported to cause acute hypoglycemia by decreasing hepatic glucose output (Randle et al., 1988), lowered systolic blood pressure, glucose level and tissue aldehyde conjugates and attenuated adverse renal vascular changes (Vasdev et al., 2000).

Vitamin E is also essential for maintaining the integrity, function and flexibility of cell membranes, Vit E is also an important fat soluble antioxidant. Tocopherol serves to detoxify and remove reactive nitrogen species (RNS) from the body. Tocotrienols work best as a team to quench the lipid and nitrogen free radicals known to cause injury to cells and tissues. Aside from its antioxidant properties, Vit E may support normal cell division and immune health, influence blood coagulation speed and provide protection to neural tissues (Skrzydlewska et al., 2001; Coulter et al., 2006). The objective of this study was to assess renal cell responses to ZnO-NPs so as to show their potential toxic biological responses and investigate the renoprotective effect of α-Lip and Vit E.

## MATERIALS AND METHODS

### Chemicals

The 50-nm ZnO powders were purchased from Sigma Co. (USA). All chemicals used were of high analytical grade, product of Sigma and Merck companies.

### Animals and treatments

Fifty Wistar albino rats weighing 180 to 200 g were used. The rats were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University. Animals have been kept in special cages and maintained on a constant 12-h light/12-h dark cycle with air conditioning and temperature ranging 20 to 22°C and humidity (60%). Rats were fed with standard rat pellet chow with free access to tap water *ad libitum* for one week before the experiment for acclimatization. Animal experimental protocols were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Ethic Committee in Research of the King Saud University, College of Pharmacy.

After one week acclimation, the rats were kept fasting over night before treatment and randomly divided into two classes according to the dose of ZnO-NP administered to rats. Class I consisted of four groups (ten rats per group): G1: normal healthy animals; G2 - G4: animals orally administered 600 mg/kg body weight/day ZnO-NP for 5 days (Wang et al. 2008a) and divided as follows: G2: ZnO-intoxicated animals with a low oral dose (600 mg/kg/day) daily for 5 days; G3: ZnO-intoxicated animals co-administered α-Lip (200 mg/kg) daily (Sharma and Gupta, 2003); G4: ZnO-intoxicated animals co-administered Vit E (100 mg/kg) daily (Ishrat et al., 2009). On the other hand, Class II consisted of three groups (G5-G7; ten rats per group) orally administered 1 g/kg body weight/day for 5 days ZnO-NP (Wang et al. 2008a), and divided as follows: G5: ZnO-intoxicated animals with a high dose (1 g/kg/day) daily for 5 days; G6: ZnO-intoxicated animals co-administered α-Lip (200 mg/kg) daily; G7: ZnO-intoxicated animals co-administered Vit E (100 mg/kg) daily.

A-Lipoic acid and Vit E were orally administered daily for three

constitutive weeks from the beginning of the experiment. The body weights of rats were recorded before and after the administration period. Three weeks later and after 24 h of the last dose administration, rats were fasted overnight then sacrificed and the blood was collected. Serum was separated and kept at -80°C for different biochemical estimations. Both the kidneys were harvested through a midline incision, rinsed in cold isotonic saline, homogenized, and frozen at -80°C for estimations of GSH content. Another three kidneys from each group were kept in 4% formalin for histopathological examination.

**Serum biochemical analysis**

***Determination of TNF-α level***

TNF-α in serum was determined using commercially available enzyme-linked immunosorbent assays (ELISA) following the instructions supplied by the manufacturer (Duo Set kits, R and D Systems; Minneapolis, MN, USA). The results are shown as pg of cytokine per ml.

***Determination of C-reactive protein (CRP) level***

CRP was measured with latex-enhanced immunonephelometry on a Behring BN II nephelometer (Dade Behring). In this assay, polystyrene beads coated with rat monoclonal antibodies bind CRP present in the serum sample and form aggregates. The intensity of scattered light is proportional to the size of the aggregates and thus concentration of CRP present in the sample. The intra-assay and inter-assay coefficients of variation for CRP were 3.3 and 3.2%, respectively. The lower detection limit of the assay was 0.15 mg/L (Kim et al., 2010).

***Determination of vascular endothelium growth factor (VEGF) level***

The level of VEGF in serum was determined at 492 nm by quantitative colorimetric sandwich ELISA (R and D systems, UK) in accordance with the manufacturer's instructions (Yao et al., 2005). Concentrations were calculated using a standard curve generated with specific standards provided by the manufacturer.

***Determination of IgG level***

The IgG level was measured in serum using a sandwich ELISA. The capture antibody was goat anti-rat IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). Standards were prepared from rat IgG (Sigma Chemical Co., St. Louis, MO). Goat anti-rat IgG peroxidase conjugates were diluted at 1:250 in phosphate buffered saline/bovine serum albumin (PBS/BSA) (from Kirkegaard and Perry Laboratories, Inc.) and used as detecting antibodies. The chromogenic substrate used was 2,2′-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS; Kirkegaard and Perry Laboratories, Inc.). Color development was detected via optical density at 405 nm using an automated ELISA plate reader (Bio-tek Instruments, Inc., Winooski, VT) and immunoglobulin concentrations were determined by comparing sample color development to standard curves (Kineticalc, Bio-tek Instruments Inc.).

***Determination of interleukin-6 (IL-6) level***

IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R and D Systems, Minneapolis, MN) with an analytical CV of 6.3% and a detection level of 0.04 pg/ml (Kaden, 2007).

***Determination of nitrite level***

Nitrite level concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium (Moshage et al, 1995).

***Determination of glucose level, serum urea, creatinine and uric acid level***

Glucose level was estimated using the method of Trinder (1969). Moreover, serum was assayed for urea, creatinine, and uric acid by using standard diagnostic kits.

***Determination of GSH level in renal tissue***

Renal content of GSH was estimated according to the method of Moron et al. (1979).

**Histopathological technique**

Samples of kidney tissues were collected to be fixed in 4% formaldehyde for 24 h, and then they were dehydrated in ascending grades of ethyl alcohol, then cleared in xylene and embedded in paraffin. Paraffin blocks were cut by microtone at 4 µM, and then fixed on glass slides to be ready for staining. Subsequently, sections were stained with hematoxylin and eosin (H and E), hydrated in descending grades of alcohol, stained with hematoxylin to stain the nuclei, and then stained with eosin to stain the cytoplasm. Another set of unstained slides were stained with Masson's trichrome stain to visualize deposition of collagen fibers which usually takes the green coloration (Smith and Bruton1978).

**Statistical analysis**

Data are presented as the mean ± S.D. Statistical analysis was performed using Instat-3 computer program (Graph pad software Inc, San Diego, CA, USA). One way analysis of variance (ANOVA) followed by Bonferroni multiple tests was used to determine the differences between means of different groups. The level of significance was set at $p \leq 0.05$.

## RESULTS

The current investigation revealed that ZnO-NP treatments either in low or high dose did not affect neither body weight nor kidney weight compared to control group. Also, administration of the two antioxidants had no effect on body or kidney weights (Tables 1 and 2). Oral administration of the two doses of ZnO-NP significantly elevated NO, glucose and IgG serum levels, whereas the elevation in VEGF level was dose-dependent compared to normal control values (Figures 1 and 3). Administration of either α-Lip or Vit E markedly down regulated the previous elevated biomarkers. Moreover TNF- α, IL6 and CRP serum levels were significantly elevated in both low

**Table 1.** Effect of α –Lip or Vit E treatments on body weight, kidney weight, and kidney/body weight % in intoxicated rats with small dose of ZnO-NP particles.

| Groups | Body Weight (g) | | Kidney Weight (g) | Kidney /Body Weight % |
|---|---|---|---|---|
| | Initial | Final | | |
| Control | 240.6 ± 8.39 | 266 ± 24.73 | 1.23 ± 0.154 | 0.443 ± 0.190 |
| ZnO-NP | 254.4 ± 15.15 | 279.6 ± 9.50 | 1.32 ± 0.214 | 0.415 ± 0.044 |
| α –Lip | 249.4 ± 14.84 | 277 ± 20.30 | 1.31 ± 0.262 | 0.493 ± 0.088 |
| Vit E | 248.2 ± 12.27 | 271.8 ± 19.66 | 1.22 ± 0.092 | 0.499 ± 0.064 |

Data are presented as mean ± SD of 10 rats.

**Table 2.** Effect of α –Lip or Vit E treatment on body weight, kidney weight and Kidney/body weight % in intoxicated rats with high dose of ZnO-NP particles.

| Groups | Body Weight (g) | | Kidney Weight (g) | Kidney/Body Weight % |
|---|---|---|---|---|
| | Initial | Final | | |
| Control | 256 .9 ± 8.38 | 281.8 ± 20.82 | 1.23 ± 0.130 | 0.485 ± 0.088 |
| ZnO-NP | 235.5 ± 18.66 | 266.6 ± 25.83 | 0.93 ± 0.26 | 0.366 ± 0.151 |
| α –Lip | 250.4 ± 20.30 | 282.8 ± 38.85 | 1.33 ± 0.236 | 0.477 ± 0.088 |
| Vit E | 245 ± 20.58 | 270.6 ± 18.57 | 1.17 ± 0.333 | 0.428 ± 0.044 |

Data are presented as mean ± SD of 10 rats.

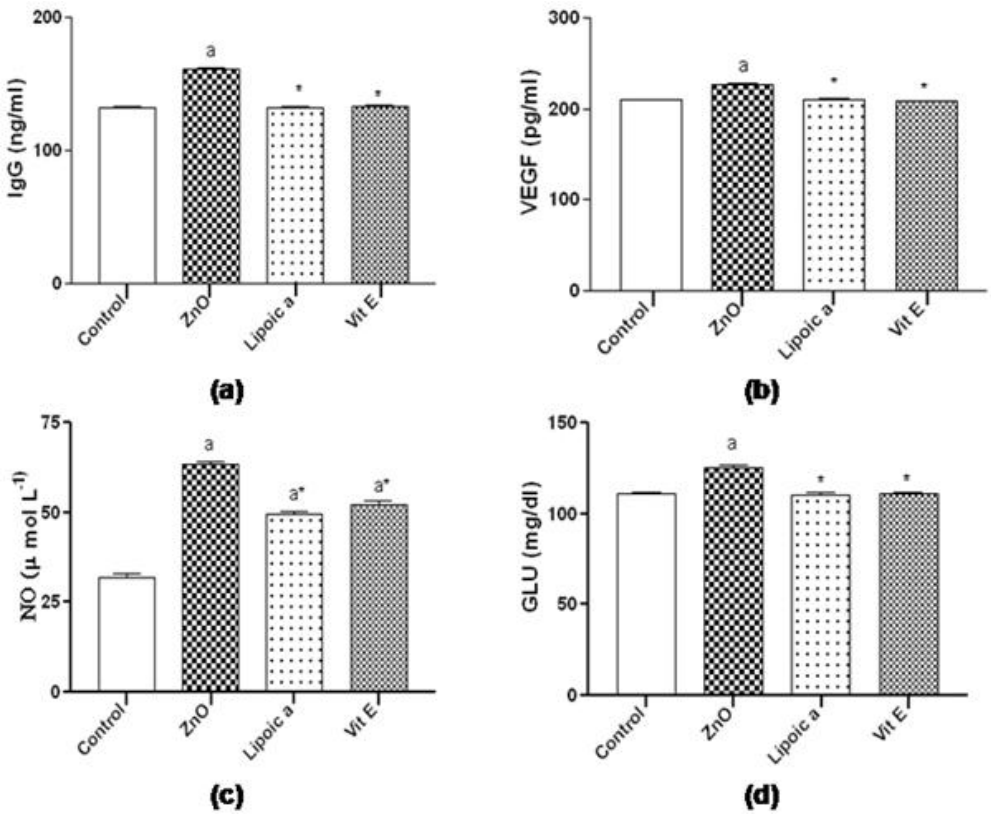


**Figure 1.** Effect of α-Lip or Vit E treatments on serum IgG (a), VEGF (b), total nitrate/nitrite (c) and glucose (d) levels in intoxicated rats with small dose o ZnO-NP particles. Values are expressed as mean ± S.E. $^{a}P < 0.001$ compared to normal control group, *P < 0.001 compared to ZnO-NP intoxicated group respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

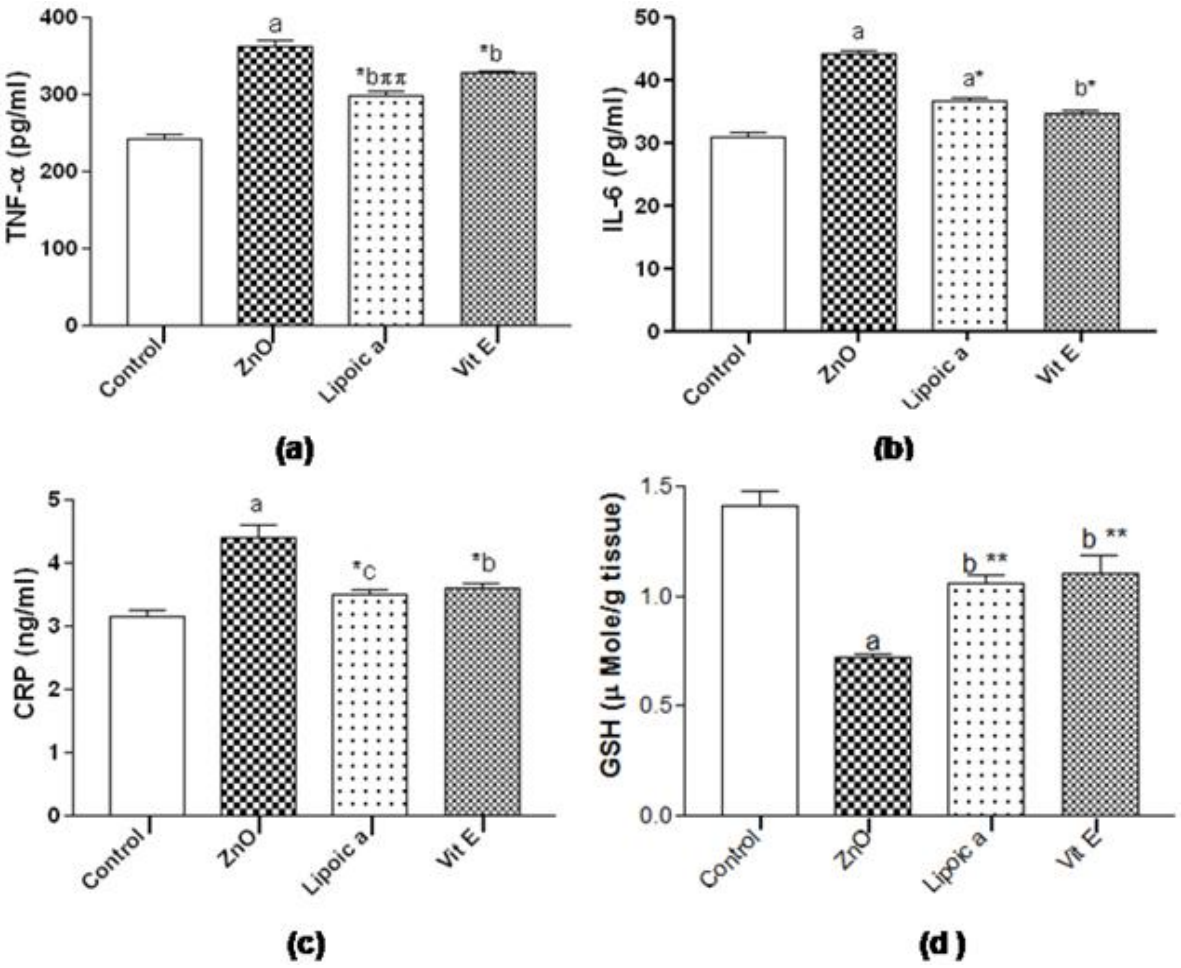


**Figure 2.** Effect of α-Lip or Vit E treatment on serum TNF-α (a), IL-6 (b), CRP (c), and GSH (d) levels in intoxicated rats with small dose of ZnO-NP. Values are expressed as mean ± S.E. $^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$ compared to normal control group, *P<0.001 ,**P<0.01 compared to ZnO-NP intoxicated group, $^{\pi\pi}P < 0.001$ compared with vitamin E group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

and high doses ZnO-NP treatments compared to untreated group. A-Lipoic acid or Vit E treatments reduced these elevated parameters (Figures 2 and 4). On the other hand, renal GSH level decreased upon ZnO-NP treatments. Administration of either α-Lip or Vit E ameliorated the reduced GSH level (Figures 2 and 4). There was also a significant increase in urea and creatinine levels in serum of ZnO-NP intoxicated rats compared to normal control group ($p$<0.05) (Tables 3 and 4). Vitamin E significantly decreased serum creatinine level compared to rats intoxicated with high dose of ZnO-NP ($p < 0.05$).

In general, it is obvious that α-Lip and Vit E have nearly the same antioxidant effect. The previous biochemical parameters were supported by the histopathological examination which revealed that kidney sections stained with H and E treated with high dose of ZnO-NP showed massive atrophy and fragmentation of numerous glomeruli. In addition the renal tubules showed epithelial exfoliation, degeneration and necrosis. Some of renal tubules showed casts in their lumina. Severe congestion was observed in renal interstitium (Figure 5B). Treatment with α-Lip showed moderate histopathological changes in the form of shrinkage and fragmentation of few glomeruli with exfoliation of tubular epithelial cells and tubular casts in few renal tubules (Figure 5C). Animals that received ZnO-NP and Vit E showed histopathological changes in the form of marked hyperplasia of glomerular mesangial cells in many glomeruli, obliteration of many capsular spaces and necrosis and exfoliation of tubular epithelial cell lining of few renal tubules (Figure 5D).

On the other hand, Kidney sections stained with Masson's trichrome, of control group showed minimum amount of collagen fibers in the form of thin rim in between the renal tubules in the interstitial tissue (Figure 6A). While animals receiving high dose of ZnO-NP showed marked increase of deposition of collagen fibers

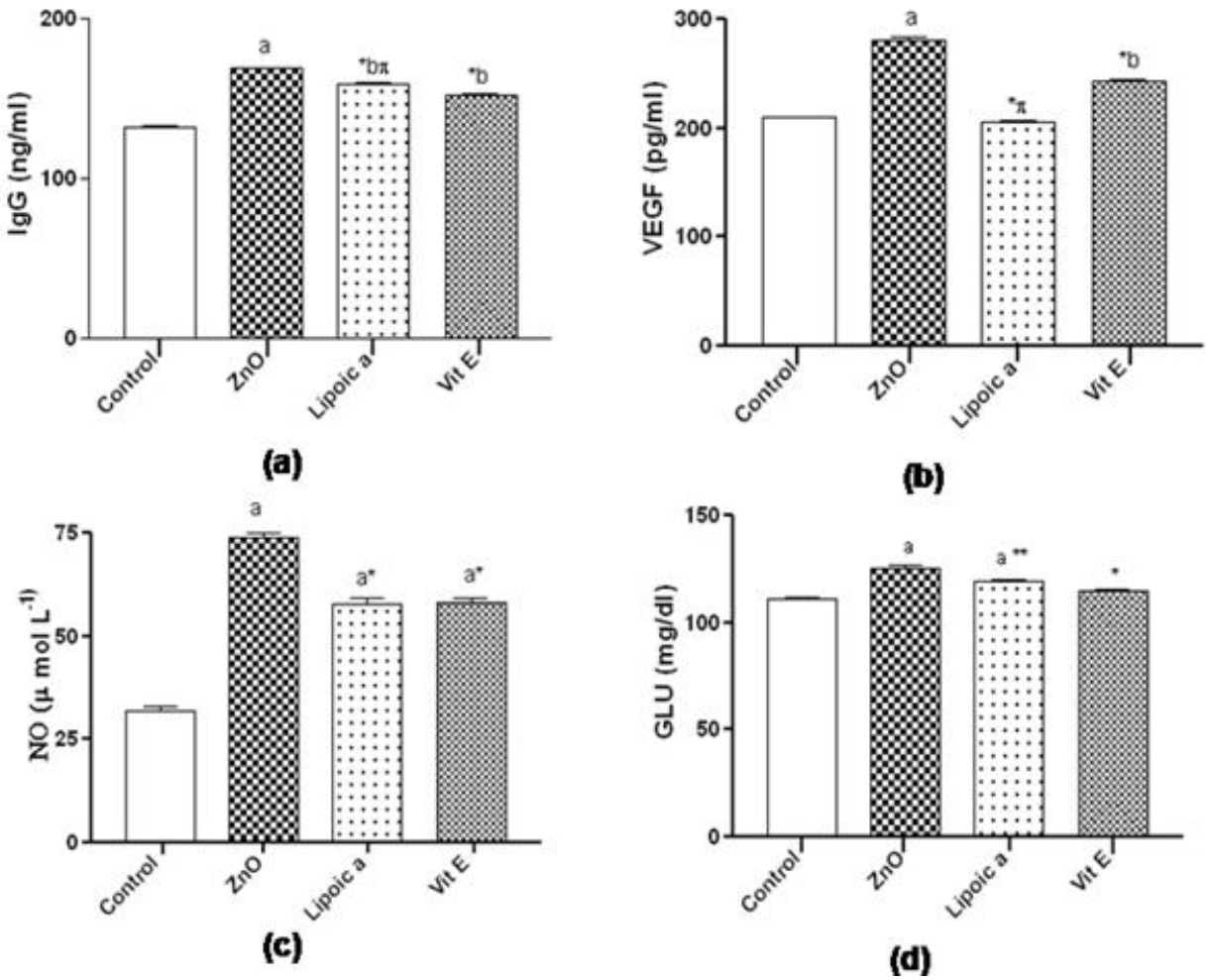


**Figure 3.** Effect of α-Lip or Vit E treatment on serum IgG (a), VEGF (b), total nitrate/nitrite (c), and glucose (d) levels in intoxicated rats with large dose of ZnO-NP particles. Values are expressed as mean ± S.E. $^{a}P < 0.001$, $^{b}P < 0.01$ compared to normal control group, *P < 0.001, **P < 0.01 compared to ZnO-NP intoxicated group, $^{\pi}P \leq 0.05$ compared with vitamin E group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

in the interstitial tissue (Figure 6B), administration of α-Lip along with ZnO-NP showed mild increase of collagen deposition (Figure 6C). Vitamin E with ZnO-NP showed moderate increase of collagen deposition (Figure 6D). Animals that received low dose of ZnO-NP showed either atrophy and fragmentation or mesangial hyperplasia of few glomeruli. However, many renal tubules showed epithelial exfoliation, degeneration and necrosis. In addition, few renal tubules showed casts in their lumina. Severe congestion was observed in renal interstitium (Figure 7B). Co-administration of ZnO-NP and α-Lip produced a shrinkage and fragmentation of few glomeruli with necrosis and exfoliation of tubular epithelial cells and tubular casts in few renal tubules (Figure 7C). In addition, animals that received ZnO-NP and Vit E showed moderate mesangial hyperplasia in many glomeruli with necrosis and exfoliation of tubular epithelial cells and tubular casts in few renal tubules (Figure 7D). Animals that received low dose of ZnO-NP showed marked increase of collagen fibers in the interstitial tissue (Figure 8B). ZnO-NP and α-Lip and or Vit E administration showed mild increase of collagen deposition (Figure 8C and 8D), respectively. Pathological changes of low-dose group are less than those of high dose group; also collagen deposition in low-dose group is much less than high dose group.

## DISCUSSION

Nanoparticles are known to disseminate to several organs such as liver, spleen, kidneys, brain or heart (Oberdorster et al., 2005; Jain et al., 2008). Kidneys play an important role in eliminating xenobiotics from the body, and thus NPs absorbed in the systemic circulation can be excreted by renal clearance (Schipper et al., 2009). Until now, little attention has been paid to renal cells as a target for NP toxicity. This study aimed to

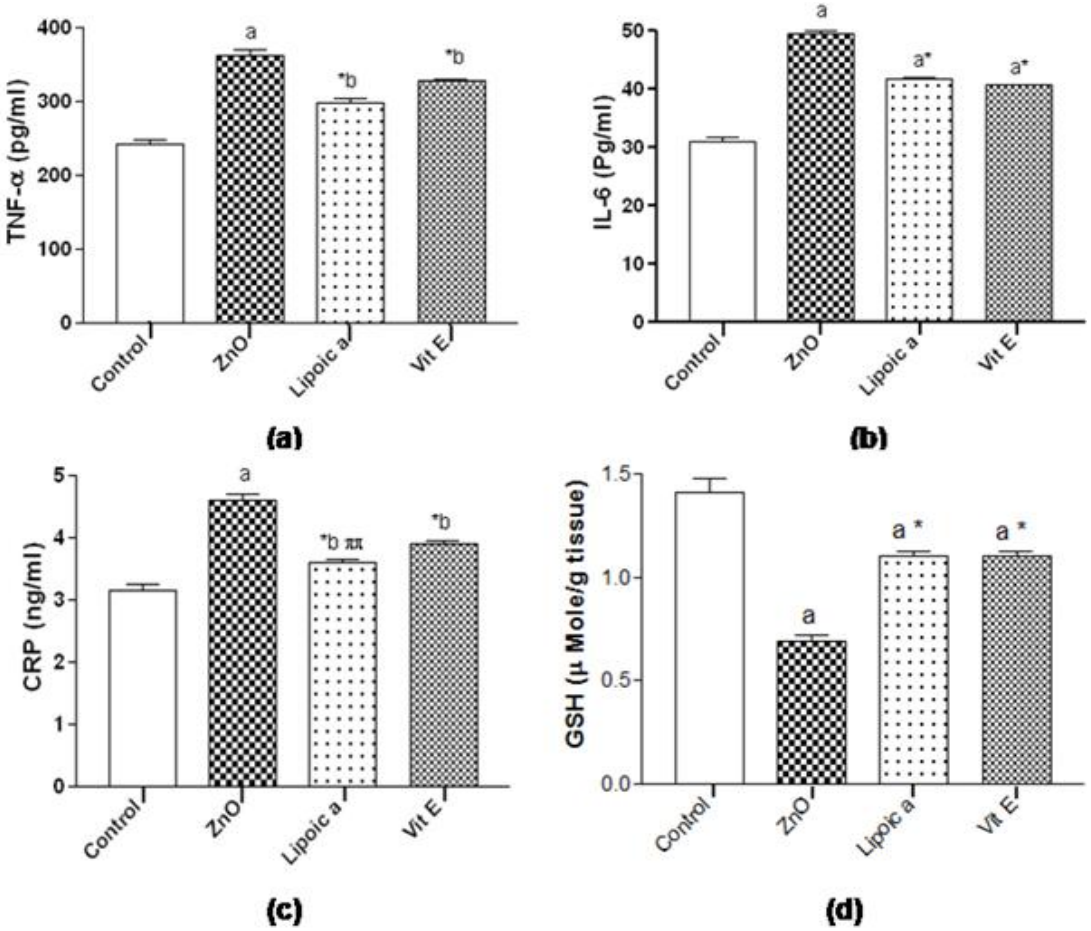


**Figure 4.** Effect of α-Lip or Vit E treatments on serum TNF-α (a), IL-6 (b), CRP (c), and GSH (d) levels in intoxicated rats with large dose of ZnO-NP particles. Values are expressed as mean ± S.E. [a]P<0.001, [b]P<0.01 compared to normal control group, *P<0.001 compared to ZnO-NP intoxicated group, ππP < 0.001 compared with Vit E group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

**Table 3.** Effect of α–Lip or Vit E treatment on serum creatinine, urea and uric acid levels in intoxicated rats with low dose of ZnO-NP.

| Parameters | Control | ZnO-NP | α –Lip | Vit E |
|---|---|---|---|---|
| Creatinine(mg/dl) | 0.5 ± 0.077 | 0.61 ± 0.087 | 0.57 ± 0.079 | 0.53 ± 0.026 |
| Urea(mg/dl) | 22.4 ± 5.01 | 33.3 ± 7.03 [a] | 29.6 ± 5.77 | 28.8 ± 2.82 |
| Uric acid(mg/dl) | 2.2 ± 0.35 | 2.72 ± 0.60 | 2.54 ± 0.66 | 2.34 ± 0.51 |

Data are presented as mean ± S.D. of 10 rats, [a] $P \leq 0.05$ compared with normal group using ANOVA followed by Bonferroni as a post-ANOVA test.

**Table 4.** Effect of α–Lip or Vit E treatment on serum creatinine, urea and uric acid level in intoxicated rats with high dose of ZnO-NP particles.

| Parameters | Control | ZnO-NP | α –Lip | Vit E |
|---|---|---|---|---|
| Creatinine (mg/dl) | 0.5 ± 0.073 | 0.73 ± 0.037 [a] | 0.61 ± 0.077 | 0.60 ± 0.044 [*] |
| Urea (mg/dl) | 20.4 ± 5.01 | 34.3 ± 5.77 [b] | 28.9 ± 4.66 | 27.97 ± 2.05 |
| Uric acid (mg/dl) | 2.2 ± 0.56 | 3.12 ± 0.56 | 2.94 ± 0.88 | 2.99 ± 0.60 |

Data are presented as mean ± S.D. of 10 rats, [a] $P \leq 0.001$, [b] $P \leq 0.01$, [c] $P \leq 0.05$ compared with normal group, [*] $P \leq 0.05$ compared with ZnO-NP intoxicated group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test

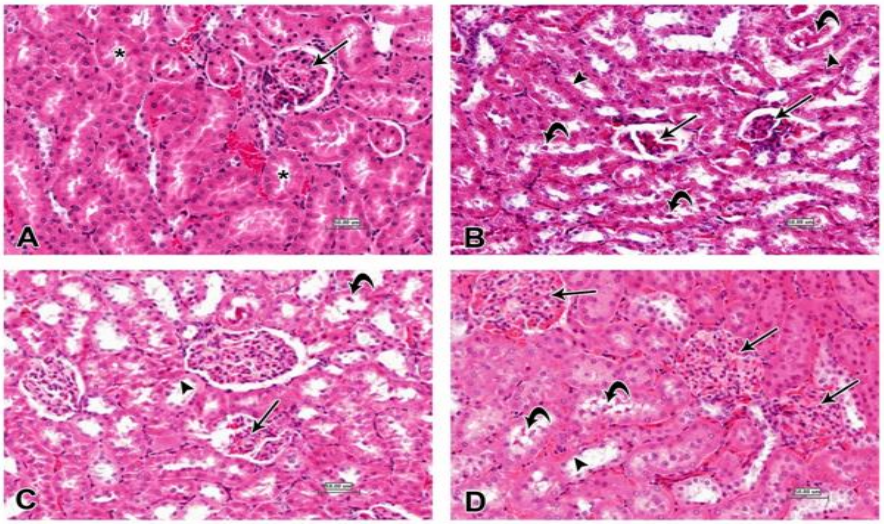


**Figure 5.** Photomicrographs of kidney sections stained with H and E from animals received high dose of nZnO. Scale bar= 50 µM. (A) Kidney from control animal showing normal renal corpuscle ( arrow) and renal tubules (asterisk). (B) Kidney from animal that received high dose of ZnO-NP showing marked shrinkage and fragmentation of glomeruli (arrow) and necrosis (arrow head) and exfoliation (curved arrow) of epithelial cell lining of many renal tubules. (C) Kidney from animal that received high dose of ZnO-NP and α-Lip acid showing shrinkage and fragmentation of few glomeruli (arrow) and necrosis (arrow head) and exfoliation (curved arrow) of epithelial cell lining of few renal tubules. (D) Kidney from animal that received high dose of ZnO-NP and Vit E showing marked hyperplasia of glomerular mesangial cells in many glomeruli (arrow) with obliteration of capsular spaces. Few renal tubules show necrosis (arrow head) and exfoliation (curved arrow) of their epithelial cell lining.

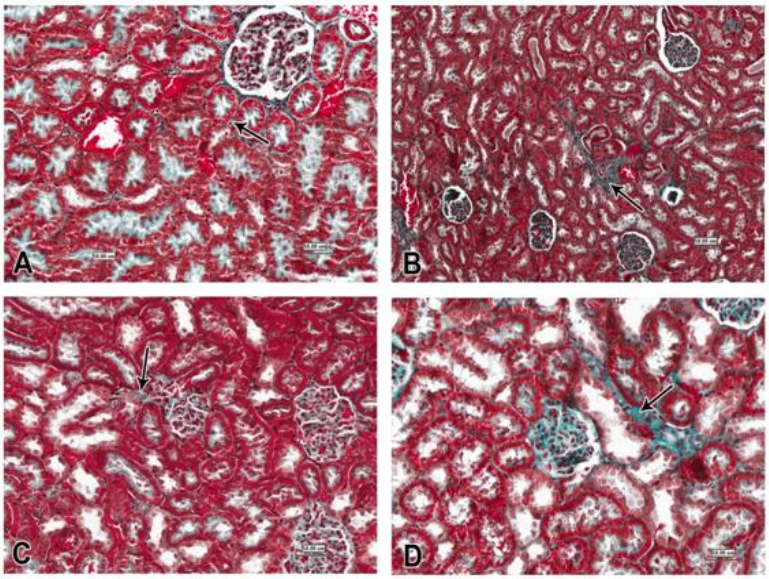


**Figure 6.** Photomicrographs of kidney sections stained with Masson's trichrome from animals received high dose of nZnO. Scale bar=50 µM. (A) Kidney from control animal showing thin rim of collagen fibers (arrow) in between renal tubules. (B) Kidney from animal that received high dose of ZnO-NP showing marked increase of collagen fibers deposition (arrow). (C) Kidney from animal that received high dose of ZnO-NP and α-Lip showing mild increase of collagen fibers deposition (arrow). (D) Kidney from animal that received high dose of ZnO-NP and Vit E showing moderate increase of collagen fibers deposition (arrow).

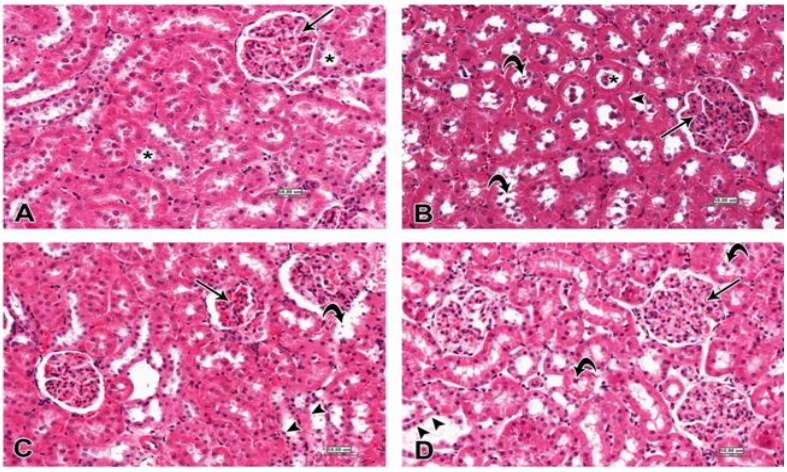


**Figure 7.** Photomicrographs of kidney sections stained with H and E from animals received low dose of nZnO. Scale bar = 50 µM. (A) Kidney from control animal showing normal renal corpuscle (arrow) and renal tubules (asterisk). (B) Kidney from animal that received low dose of ZnO-NP, showing mesangial hyperplasia in few glomeruli (arrow) and necrosis (arrow head) and exfoliation (curved arrow) of epithelial cell lining of many renal tubules. Few tubules show casts (asterisks). (C) Kidney from animal that received low dose of ZnO-NP and α–Lip showing shrinkage and fragmentation of few glomeruli (arrow) and necrosis (arrow head) of epithelial cell lining of few renal tubules. (D) Kidney from animal that received low dose of ZnO-NP and Vit E showing moderate hyperplasia of glomerular mesangial cells in many glomeruli (arrow). Few renal tubules show necrosis (arrow head) and exfoliation (curved arrow) of their epithelial cell lining.

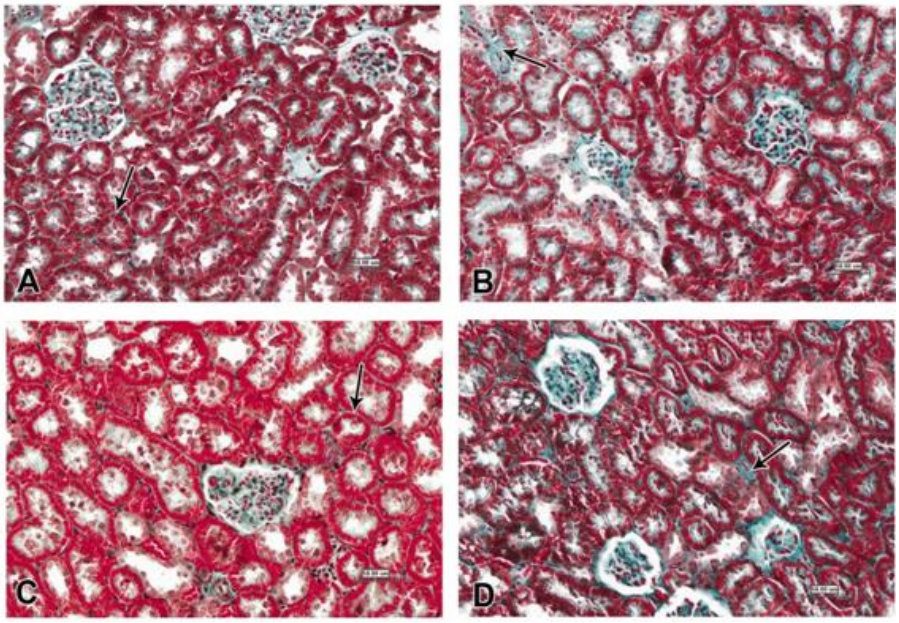


**Figure 8.** Photomicrographs of kidney sections stained with Masson's trichrome from animals received low dose of nZnO. Scale bar=50 µM. (A) Kidney from control animal showing thin rim of collagen fibers (arrow) in between renal tubules. (B) Kidney from animal that received low dose of ZnO-NP showing marked increase of collagen fibers deposition (arrow). (C) Kidney from animal that received low dose of ZnO-NP and α-Lip showing mild increase of collagen fibers deposition (arrow). (D) Kidney from animal that received low dose of ZnO-NP and Vit E showing mild increase of collagen fibers deposition (arrow).

investigate human renal cell responses to manufactured NPs in order to highlight their potential toxicity and/or biological responses, and investigate the effect of Vit E or α –Lip treatment on ZnO-NPS induced renal cytotoxicity.

In the present work, ZnO-NP significantly elevated the levels of TNF-α, IL-6 and VEGF compared to normal control group. This is in agreement with the study of Tsuo et al. (2010) who clarified the inflammatory effects of ZnO-NP particles on vascular endothelial cells, revealing that ZnO-NP particles induced a dose-dependent increase in the expression of intercellular adhesion molecule-1 (ICAM-1), an indicator of vascular endothelium inflammation, protein expression and marked increases in NF-κB reporter activity. Additionally, TNF-α, a typical inflammatory cytokine, induced ICAM-1 expression in an NF-κB-dependent manner, and ZnO-NP synergistically enhanced TNF-α-induced ICAM-1 expression of vascular disease. In the present study, the level of TNF-α was reduced post α–Lip treatment as compared to ZnO-NP treated group. These results were in accordance with a previous study in which α-Lip strongly inhibited TNF-α and induced mRNA expression of monocyte chemo attractant protein-1 (Packer et al, 1995). Furthermore, α-Lip dose-dependently inhibited TNF- α -induced I kappa B kinase activation**,** subsequent degradation of I kappa B.

Nitric oxide (NO) is a chemical mediator involved in the maintenance of physiological homeostasis due to its regulatory and protective functions. Besides its known antioxidant property, NO which is produced by inducible nitric oxide synthase (iNOS) can be cytotoxic, especially at higher local concentrations. Also, it can react with reactive oxygen species (ROS) or oxygen yielding reactive nitrogen species (RNS), which causes damage on biological molecules such as enzymes, lipids and DNA by nitrosation, oxidation and nitration. Mesangial and invading immune cells are capable of expressing iNOS upon stimulation with TNF-α, IL-1b and bacterial lipopolysaccharide (LPS), and thus are likely to be responsible for the release of large amounts of NO during TNF α, IL-1b and LPS-triggered inflammatory conditions in the glomerulus cells (Aiello et al., 1998). In the present study, ZnO-NP produced an elevation of IL-6 and nitric oxide levels, and such elevation may be due to increased expression of neuronal NOS (nNOS) mRNA and NOS activity. This was confirmed with a previous study which revealed that exposure to low concentrations of ZnO-NP elevated circulating levels of IL-6, and that could account for the symptoms of the metal fume fever syndrome (Fine et al., 1997). Administration of either Vit E, or α-Lip along with ZnO-NP produces a significant decrease in IL-6 as well as NO levels. In accordance with our results, Kielstein et al (2002) reported a reduction in NO level post Vit E treatment in chronic kidney disease patients.

Vascular endothelial growth factor (VEGF), which is a potent mitogen for endothelial cells, has been reported to be expressed in several tissues, including kidney.

Besides its mitogenic properties, VEGF is able to promote angiogenesis-induce proteases (Drexler, 1994) and increase vascular leakage. In the present study the level of VEGF was elevated post ZnO-NP treatment compared to normal group. The reduction in VEGF level in ZnO-NP along with α-Lip treatment compared to ZnO-NP treated group was confirmed with the study of Moore et al. (2009), who observed that α-lip effectively prevented Ang II-induced glomerular and vascular damage in the kidneys and completely prevented the development of albuminuria through its anti-inflammatory/antioxidative mechanisms. The effects are associated with decreased nuclear factor (kappa) B (NF-κB) and activator protein-1 (AP-1) activation, as well as improved thiol homeostasis. Ang II–induced leukocyte infiltration and cell proliferation in the kidney were attenuated. The redox-sensitive transcription factors NF-κB and AP-1 in the kidneys were increased, and were effectively reduced post α–Lip administration.

Renal oxidized GSH levels were much higher, while the opposite was true for cysteine levels. These results suggested increased renal glutathione oxidation, leading to cysteine shortage. α-Lipoic acid partly prevented renal cysteine depletion and increased hepatic cysteine and glutathione concentrations. This effect was accompanied by increased hepatic gamma-glutamyl cysteine synthetase mRNA expression, with decreased NF-κB and AP-1 activation. α–Lipoic acid can regenerate vitamin C from its oxidized form, dehydroascorbic acid and regenerate other antioxidants, as well as chelates transition metal ions (e.g. iron and copper). it can enhance the synthesis of glutathione, the main antioxidant within our cells (Randle et al., 1988; Vasdev et al., 2000). Glutathione effectively mops up all types of toxins and free radicals. It can even pitch in and help when the body is lacking Vit E. Previous results have explained the modulatory effect in GSH level in animals treated with either α–Lip or Vit E post ZnO-NP administration. Vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, the resulting antioxidant radicals being a relatively unreactive species (Ramos et al., 2011). In many studies Vit E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect (Kalender et al., 2004; Suna et al., 2004). It is concluded that Vit E is an essential component of the kidney for the protection of this tissue against peroxidative damage (Al-Attar, 2011).

In the present study, it was found that the level of pro-inflammatory biomarkers including CRP was elevated markedly in rat sera intoxicated with either two doses of ZnO-NP in relation to normal group implying immune disorder. Elevation of CRP post ZnO-NP exposure compared to normal control group was confirmed with the study of Kim et al. (2010) that clarify the increased CRP level with inflammation. In the present study, CRP level

was reduced post Vit E treatment compared to ZnO-NP exposed groups which was coincide with previous studies which revealed that Vit E plays a major role in reducing inflammation as well as cleansing the body of free radicals. As vitamin E supplements lowered CRP and IL6 concentrations dramatically in diabetic people (Singh et al, 2005; Upritchard et al., 2000). Oxidative stress and acute phase inflammation are now recognized to be highly prevalent in both the chronic kidney disease (CKD; pre-dialysis) and end stage renal disease (ESRD); on hemodialysis populations and several lines of evidence point to their contribution in the development of atherosclerosis. Biomarkers of the inflammatory state such as CRP and IL-6 are robust predictors of cardiovascular events and death in these two populations. The uremic state is characterized by retention of oxidized solutes including reactive aldehyde groups and oxidized thiol groups. It has recently been demonstrated that administration of antioxidant therapy such as Vit E and or α-Lip will decrease biomarkers of acute phase inflammation and oxidative stress in these patients (Suna et al., 2004).

The marked increase in circulating IgG in rat sera intoxicated with both doses of ZnO-NP is another response to immune disorder induced by this NPs toxicity in the current work. It was suggested that the increase in the circulating antibody production is the result of production of different inflammatory cytokines including TNF-α with potential impact on immunoglobulin production during inflammation. These results may indicate that ZnO-NP induced inflammatory kidney injury through production of the inflammatory mediators (Davis et al., 1998). IgG level was reduced by either Vit E or α-Lip treatments and this may explain the role of these agents to suppress the release of inflammatory mediators. There was also a significant increase in urea and creatinine levels in serum of ZnO-NP intoxicated rats confirmed by its nepherotoxic effect, compared to normal control group ($p < 0.05$) (Tables 3 and 4). Vitamin E significantly decreased serum creatinine level compared to rats intoxicated with high dose of ZnO-NP ($p < 0.05$). Serum glucose level was downregulated by the administration of either Vit E or α-Lip along with ZnO-NP compared to ZnO-NP treated group, and this coincide with Wang et al. (2008a) who observed that nano particles affects the pancreas. Randle et al. (1988) also reported that α-Lip causes acute hypoglycemia by decreasing hepatic glucose output.

In the present study, renal histopathological examination revealed that there was alteration of proteinaceous casts in the tubules and renal tubular dilatation in the ZnO-NP treated rats. High dose of ZnO-NP showed massive atrophy and fragmentation of numerous glomeruli, the renal tubules showed epithelial exfoliation, degeneration and necrosis. Some of renal tubules showed casts in their lumina. Severe congestion was observed in renal interstitium (Figure 5B). Treatment with α-Lip showed moderate histopathological changes in the form of shrinkage and fragmentation of few glomeruli with exfoliation of tubular epithelial cells and tubular casts in few renal tubules (Figure 5C). Animals that received ZnO-NP and Vit E showed histopathological changes in the form of marked hyperplasia of glomerular mesangial cells in many glomeruli, obliteration of many capsular spaces and necrosis and exfoliation of tubular epithelial cell lining of few renal tubules (Figure 5D). Animals that received high dose of ZnO-NP showed marked increase of deposition of collagen fibers in the interstitial tissue (Figure 6B). This histopathological change coincided with the results of Wang et al. (2006) study. Administration of α-Lip along with ZnO-NP showed mild increase of collagen deposition (Figure 6C). Moreover, vitamin E with ZnO-NP showed moderate increase of collagen deposition (Figure 6D). This can be attributed the role of Vit E or α-Lip to suppress the release of inflammatory mediators.

## Conclusion

This study highlights the nepherotoxic effect of low and high doses of ZnO-NP. This was confirmed by the significant increase in urea and creatinine levels in serum of ZnO-NP intoxicated rats. Moreover, TNF-α, IL-6 and CRP levels were significantly increased, and that contributed to the nephrotoxic potential of ZnO-NP. Treatment with either Vit E or α-Lip successively alleviated the alterations in TNF-α, IL-6 and VEGF, as well as effectively ameliorated the histopathological changes of ZnO-NP intoxicated rats. Moreover, these antioxidants markedly reduced inflammatory cytokines levels. This may be related to their ability to attenuate the extent of NO synthesis. Our data demonstrated that α–Lip and Vit E are potent antioxidants that protect renal cells from injury caused by ROS oxidative stress and related vascular complications induced by ZnO-NP. Further studies are needed to evaluate the synergistic combination of Vitamin E and α-Lip, which is known to have an additional potential concern in ameliorating ZnO-NP nepherotoxic effect.

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