

Potential prophylactic impact of B vitamins against zinc oxide bulk and its nanoparticles induced kidney damage

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Abstract: The protective effect of B vitamins combination against zinc oxid bulk (ZnO-bulk) and its nanoparticles (ZnO-NPs) toxicity -induced kidney damage in rats was investigated. ZnO- bulk or its NPs were administered orally (500 mg /kg body weight) for 10 consecutive days. The results revealed that oral co-ingestion of of B vitamins combination (250 mg B₃, 60 mg B₆ and 0.6 mg B₁₂ / Kg body weight) daily for 3 weeks to rats intoxicated by either ZnO- bulk or its NPs markedly ameliorated increases in serum markers of kidney function, including uric acid and creatinine. Also the used vitamins in combination down-modulated ZnO caused dramatic increases in serum pro-inflammatory biomarkers including, tumor necrosis factor- α (TNF- α), and C-reactive protein (CRP) as well as in serum vascular endothelial growth factor (VEGF) (angiogenic factor) in intoxicated rats compared with intoxicated untreated ones. In addition, the result showed that B vitamins effectively ameliorated the increased malondialdehyde (MDA, a major product of lipid peroxidation), the decrease in antioxidant enzyme, glutathione peroxidase (GPx), oxidative deoxyribonucleic acid (DNA) damage and the increase in the apoptosis marker caspase 3 in kidneys of intoxicated rats with either ZnO- bulk or its NPs compared with intoxicated untreated. In conclusion, prophylactic treatment with the current used B vitamins in combination may be beneficial in protecting kidney tissue from the toxic impact of ZnO- bulk or its NPs.

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1. Introduction

With the increased application of nanomaterials, the discharge to environment through production, transportation, storage, and consumption process could be rapidly increased and it may exhibit adverse effects to human health. When inhaled, nanoparticles are efficiently deposited into lung cells. Translocation through epithelial and endothelial cells into the blood and lymph circulation may be occurred to reach potentially sensitive target sites including bone marrow, lymph nodes, spleen, heart, liver and, kidneys (Oberdorster et al., 2005; Wang et al., 2007, Liu et al., 2009).

ZnO nanoparticles (ZnO-NPs) are widely applied in optoelectronics, cosmetics, catalysts, ceramics, pigments, etc. (Wang, 2004). Some studies proposed the bio-safety of ZnO and could be used in biomedical materials (Berube, 2008). However, other studies proved its adverse toxic impacts on human health and environmental species. Previous studies demonstrated that exposure to nano-ZnO resulted in oxidative stress, tissue damage and inflammatory response in vascular/lung endothelial cells (Gojova et al., 2007; Lin et al., 2009). Also, experimental studies indicated that the toxicity of ZnO-NPs affected the body vital organs including kidney (Wang et al., 2008; Fadda et al, 2012, 2013). Generally, it was demonstrated that nano-particles are more toxic than their bulk form due to their ability to penetrate into

and accumulate within cells and organisms (Ispas et al., 2009; Mironava et al., 2010).

Protective strategies against the damaging impact of metal oxide using agents with antioxidant and anti-inflammatory properties may be effective in interfering with tissue damage induced by toxicants.

Vitamin B₃ (nicotinamide) is essential to all living cells. Vitamin B₃ is biosynthetically converted to nicotinamide adenine dinucleotide (NAD), the acceptor of hydride equivalents to form the reduced dinucleotide, NADH which plays a vital role in cellular energy metabolism. In addition to being nutrients, vitamin B₃ is clinically applied pharmacological agents. Ingestion of vitamin B₃ in large doses was found to be effective in lowering serum lipids and cholesterol (Schachter, 2005). It also used for prevention of type 1 diabetes (Gale et al., 2004), and neurotoxicity and for treatment of ischemia. It has also anti-inflammatory, antioxidant (Biedron et al., 2008; Lappas and Permezel, 2011), hepatoprotective (Chen et al., 2008), and antiulcer (Abdallah, 2010) properties. Another study also revealed that vitamin B₃ ingestion resulted in a marked reduction of the pro-inflammatory mediators including TNF- α , IL-6 and the chemokine, IL-8 as well as of oxidative stress induced by of lipopolysaccharides (LPS) (Lappas and Permezel, 2011). The vitamin is benefit in increasing gene expression of superoxide dismutase (SOD), glutathione peroxidase

(GPx) and catalase (CAT) (Lappas and Permezel, 2011). Vitamin B₃ deficiency was reported to be associated with an increased risk of cancer (Kirkland, 2003). Vitamin B₃ administration is associated with increased NAD which has an important role in genome stability (Kirkland, 2003).

Vitamin B₆ (pyridoxal, pyridoxine and pyridoxamine) is involved in a number of metabolic reactions, most of which are involved in the metabolism of amino acids and proteins, lipids, carbohydrates, nucleotide, protein synthesis and cellular proliferation (Depeint et al., 2006). The vitamin has important role in the treatment of some diseases including diabetes (Jain, 2007), epilepsy (Gaby, 2007) and cardiovascular disease (Wierzbicki, 2007). Vitamin B₆ has antioxidant and radical scavenging properties (Mahfouz and Kummerow, 2004). Vitamin B₆ deficiency has been shown to lead to fatty liver (Selvam and Ravichandran, 1991); formation of calcium oxalate stones in the kidney (Selvam and Ravichandran, 1991); carcinogenesis (Reynolds, 1986) and ischaemic heart disease (Vermaak et al., 1987).

Vitamin B₁₂ (cyanocobalamin) is required for the synthesis of methionine and S-adenosyl thionine, the common methyl donor require for the maintenance of methylation patterns in DNA that determine gene expression and DNA confirmation (Zingg and Jones 1997; Solomon, 2007). It has principle roles in the treatment of different pathological conditions. Vitamin B₁₂ has anti-inflammatory, immunomodulatory, antioxidant and antioxidative stress potential actions (Miller, 2002, Wheatley, 2006, Scalabrino et al., 2008). Cbl therapy normalizes levels of TNF- α and epidermal growth factor in Cbl-deficient patients (Scalabrino et al., 2008). It acts as a second-line of defense when O₂^{•-} production overwhelms the superoxide dismutase (SOD) protection system (Moreira et al., 2011). The reduced form of Cbl, cob (II) can scavenge O₂^{•-} which is an important mechanism by which Cbl can protect cells against oxidative stress (Moreira et al., 2009). Vitamin supplements containing cyanocobalamin (CNCbl, vitamin B₁₂) decrease low-density lipoprotein oxidation in both healthy individuals and patients with coronary artery disease (Earnest et al., 2003). High doses of Cbl have been used to treat pernicious anemia for decades with no apparent toxicity (Mangiarotti, 1986).

The objective of the present study is to explore the potential prophylactic impact of combination of B vitamins (vitamins B₃, B₆ and B₁₂) in attenuating inflammation, oxidative DNA damage, oxidative stress and apoptosis induced by either ZnO – bulk or its NPs toxicity in rat kidney.

2. Materials and methods

Chemicals:

ZnO – bulk and its NPs (<100 nm) powders were purchased from Sigma Co. (USA). Vitamin B₃, B₆ and B₁₂ were purchased from Sigma–Aldrich Corporation. All other chemicals used in the study were of high analytical grade and products of the Sigma and Merck companies.

Animals and treatments:

Fifty healthy male albino rats (120–150 g) were supplied by the Experimental Animal Center, King Fahad Medical Research Center, Jeddah, King Abdelaziz University. Animal utilization protocols were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the College of Science, King Abdelaziz University. Animals were kept in special cages and maintained on a constant 12-h light/12-h dark cycle with air conditioning and a controlled temperature of 20°C to 22°C and humidity of 60%. Rats were fed a standard rat pellet chow with free access to tap water *ad libitum* for 1 week before the experiment for acclimatization. After 1 week of acclimation, the rats were randomly divided into five groups:

G1: Normal, healthy animals.

G2: ZnO- bulk intoxicated rats.

G3: ZnO-NPs intoxicated rats.

G4: ZnO- bulk intoxicated rats with co-administration of combination B vitamins

G5: ZnO-NPs intoxicated rats with co-administration of B vitamins

ZnO-bulk and ZnO-NPs were administered to rats orally (500mg/Kg body weight, Wang et al., 2008) for 10 consecutive days. They were suspended in 1% Tween 80 and dispersed by ultrasonic vibration for 15 min before administration. The control group was given 1% Tween 80 solution instead. Vitamin B₃ (250 mg Kg body weight, Godin et al. 2012), B₆ (60 mg/ Kg body weight, Macêdo et al., 2011) and B₁₂ (0.6 mg / Kg body weight, Macêdo et al., 2011) were administered orally in combination daily for three weeks. Three weeks later, the rats of all groups were kept fasting over night (12-14 h), the blood samples were collected from each animal in all groups into sterilized tubes for serum separation. Serum was separated by centrifugation at 3000 r.p.m. for 10 minutes and used for biochemical serum analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the kidney samples were collected, minced and homogenized in ice cold bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 minutes at 10000 g. at 4°C and the supernatants were used for biochemical tissue analysis.

Biochemical serum assay:

Uric acid (Fossati et al., 1980) and creatinine (Henry, 1974) were measured as indicators of kidney function. The concentration of inflammatory cytokines such as tumor necrosis factor (TNF)- α was determined using commercially available ELISA assays following the instructions supplied by the manufacturer (DuoSet kits; R&D Systems, Minneapolis, MN, USA). C-reactive protein (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 the scattered light is proportional to the size of the aggregates and thus reflects the concentration of CRP present in the sample. The level of vascular endothelial growth factor (VEGF) was assayed to be 492 nm by quantitative colorimetric sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems, UK) in accordance with the manufacturer's instructions. Concentrations were calculated using a standard curve generated with specific standards provided by the manufacturer.

Biochemical assay of kidney tissue:

Lipid peroxidation was estimated by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using extinction coefficient value(ϵ) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm).

Glutathione peroxidase assay:

GPx activity was quantified by the dithio-binitrobenzoic acid method (Rotruck et al., 1973), based on the reaction between remaining glutathione after the action of GPx and 5,5'-dithio bis-(2-nitro benzoic acid) to form a complex that absorbs maximally at 412 nm.

Assay of caspase 3 activity:

Caspase-3-like protease was assayed according to the method described by Nath et al., (1996).

Comet assay:

The comet assay, or single cell gel electrophoresis, is a widely used technique for measuring and analyzing DNA breakage in individual cells. The method of Singh et al., (1988), which involves the unwinding of DNA under alkaline conditions, was used in this study. The parameters measured to analyze the electrophoretic patterns were the tail length as measured from the middle of the head to the end of the tail and the relative DNA content in the tail. The tail moment was defined by the

percentage of DNA in the tail multiplied by the length between the center of the head and tail which was defined by Olive et al., (1990).

Statistical analysis:

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean \pm SE. Significant differences among values were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test.

3. Results

Serum kidney damage biomarkers including creatinine and uric acid in the normal and different experimental rat groups intoxicated with either repeated doses of ZnO- bulk or its NPs are shown in (Table 1). The two toxic forms of ZnO (G2 and G3 respectively) induced pronounced increases in these biomarkers compared with normal animals (G1, $P \leq 0.001$), however the deviation in these biomarkers was more pronounced in rats intoxicated with ZnO – NPs (G3). The oral ingestion of combination of B vitamins to rats intoxicated with either ZnO- bulk or its NPs (G4 and G5 respectively) significantly down-modulated the deterioration in these markers compared with either intoxicated rat group.

The levels of some immunological pro-inflammatory biomarkers, including TNF- α and CRP in different experimental rat groups are depicted in (Tables 2). These biomarkers were dramatically elevated in the sera of rats intoxicated with ZnO- bulk or its NPs versus normal group, however, the alteration in these biomarkers was more evident in ZnO-NPs intoxicated rats rat group compared with rats intoxicated with bulk ZnO. The immediate intake of vitamin B complex with ZnO ingestion markedly inhibited the induced inflammatory mediators compared with animals intoxicated with either ZnO- bulk or its NPs.

The level of VEGF (angiogenic factor) significantly increased in the sera of rats intoxicated with either ZnO- bulk or its NPs compared with normal animals (Table 2). Co- ingestion of B vitamins combination, markedly reduced the up-regulation in this factor in ZnO- intoxicated rats compared with either intoxicated untreated animal group ($P \leq 0.001$).

Table 3 shows the levels of MDA (marker of lipid peroxidation and oxidative tissue damage) and GPx (antioxidant biomarker) in rat kidneys of normal and ZnO intoxicated rats. The data revealed that toxicity of this metal oxide induced increased MDA accompanied with a decrease in the antioxidant enzyme, GPX, compared to normal animals. This effect was severe in rat kidneys ingested ZnO- NPs. Co- ingestion of B vitamins combination to rat groups intoxicated with either ZnO-bulk or its NPs effectively

ameliorated the alteration in these markers with respect to either intoxicated untreated group.

Table 3 and Figure 1 illustrate the biomarkers of DNA damage in kidneys of ZnO- intoxicated rats. A significant increases in the tail length, DNA % (tail DNA content) and tail -DNA moment were observed in rats intoxicated with either ZnO- bulk or its NPs. This effect was more evidenced in rat kidneys intoxicated with the repeated doses of ZnO-NPs. Co-administration of combination of the current vitamins,, significantly protected their kidneys from DNA

damage as indicated by a decrease in tail length, DNA % and tail -DNA moment in either ZnO intoxicated group compared with intoxicated untreated rats.

The kidney apoptosis biomarker caspase 3 was significantly up-regulated in rats administered either repeated doses of ZnO- bulk or its NPs (Table 3). Co-administration of the studied vitamins combination to either ZnO- -intoxicated rat group, beneficially down-modulated the increase in kidney caspase 3 compared with either ZnO intoxicated untreated group.

Table 1. Impact of B-vitamins on serum renal function biomarkers in ZnO intoxicated rats

Groups	Creat. mg/dl)	Uric acid (mg/dl)
Normal	0.21±0.01	0.433±0.15
ZnO-bulk	0.36±0.01 ^a	1.27±0.068 ^a
ZnO-NPs	0.45± 0.01 ^{ass}	1.5± 0.10 ^{as}
ZnO-bulk+ B Vitamins	0.23±0.01 ^{c^{ss}}	1.07±0.06 ^{as}
ZnO-NPs+ B Vitamins	0.3±0.01 ^{a^{##}}	1.13±0.057 ^{a^{##}}

Data are presented as mean ± SD of 6 rats, ^a $P \leq 0.001$, ^c $P \leq 0.05$ compared with normal group, ^s $P \leq 0.05$, ^{ss} $P \leq 0.01$ compared with ZnO-Bulk -intoxicated group, ^{##} $P \leq 0.01$ compared with ZnO-NPs intoxicated group

Table 2. Effect of B-vitamins on some serum immuno-inflammatory biomarkers (TNF- α and CRP) and angiogenic biomarker in ZnO intoxicated rats

Groups	TNF- α (pg/ml)	CRP(ng/ml)	VEGF (Pg/ml)
Normal	9.78±1.4	2.6±1.1	174.4±4.7
ZnO-bulk	33.65±1.5 ^a	16.47±0.8 ^a	204.6±3.68 ^a
ZnO-NPs	38.4±2.3 ^{ab}	19.4±1.4 ^{ass}	217.35±7.5 ^{as}
ZnO-bulk+ B Vitamins	22.6±2.3 ^{ass}	10.38± 1.02 ^{ass}	126.1±5.3 ^{ass}
ZnO-NPs+ B Vitamins	24.8±1.6 ^{a[#]}	8.5±1.12 ^{a[#]}	136.6±2.9 ^{a[#]}

Data are presented as mean ± S.D. from 6 rats, ^a $P \leq 0.001$ compared with the normal group, ^{ss} $P \leq 0.001$, ^s $P \leq 0.05$ compared with ZnO-bulk intoxicated group, [#] $P \leq 0.001$, compared with ZnO-NPs intoxicated group using ANOVA followed by Bonferroni as a post-ANOVA test

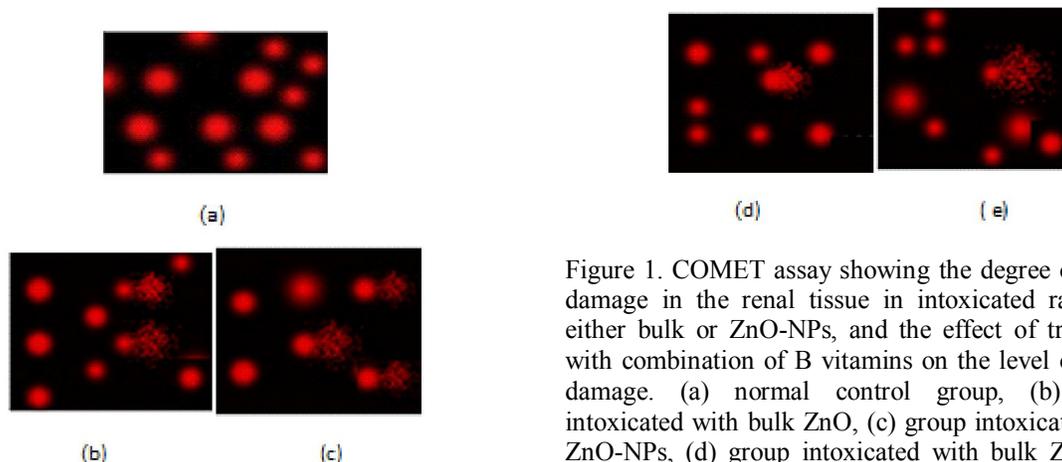


Figure 1. COMET assay showing the degree of DNA damage in the renal tissue in intoxicated rats with either bulk or ZnO-NPs, and the effect of treatment with combination of B vitamins on the level of DNA damage. (a) normal control group, (b) group intoxicated with bulk ZnO, (c) group intoxicated with ZnO-NPs, (d) group intoxicated with bulk ZnO and co-administered with B vitamins, (e) group intoxicated with ZnO-NPs and co-administered with B vitamins

Table 3. Effect of treatment with combination of B vitamins on oxidative stress, apoptosis and oxidative DNA damage biomarkers in ZnO different experimental rat groups

parameters	Normal	ZnO-bulk	ZnO-NPs	ZnO-bulk+ B vitamins	ZnO-NPs+ B vitamins
MDH(nmol/g)	4.2±0.25	7.96±0.37 ^a	9.29±0.66 ^{as}	6.0±0.44 ^{as}	6.58±0.56 ^{as}
GPX (nmol/min/mg (protein))	20.5±0.53	15.9±0.65 ^a	13.4±0.47 ^{as}	19.2±0.85 ^{css}	18.4±0.5 ^{bs}
Tail-DNA length (µm)	2.42±0.14	4.8± 0.06 ^a	5.9±0.07 ^{as}	3.1±0.10 ^{ass}	3.9±0.1 ^{as}
Tail-DNA%	2.24±0.05	4.4± 0.03 ^a	5.19±0.04 ^{ass}	3.0±0.05 ^{ass}	3.88±0.07 ^{as}
Unit Tail -DNA moment	11.4±0.011	21.6±0.85 ^a	27.14±0.98 ^{ass}	10.43±.50 ^{nss}	16.43±0.32 ^{as}
Caspase-3 (ng/g)	96.6±5.8	120.3±8.00 ^a	140.46± 6.6 ^{as}	100.23±3.7 ^{nss}	100.36±2.08 ^{as}

Data are presented as mean ± S.D. from 6 rats, ^a $P \leq 0.001$, ^b $P \leq 0.01$, ^c $P \leq 0.05$, n, nonsignificant compared with the normal group, ^{ss} $P \leq 0.001$, ^s $P \leq 0.01$ compared with ZnO-bulk intoxicated group, [#] $P \leq 0.001$, compared with ZnO-NPs intoxicated group using ANOVA followed by Bonferroni.

4. Discussion

Previous studies has been documented the reotoxicity of ZnO-NPs (Wang et al., 2008, Fadda et al., 2012).

The present study demonstrated that administration of either ZnO- bulk or its NPs (500mg/Kg body weight/day) for ten consecutive days induced nephrotoxicity in rats, as demonstrated by the significantly increased levels of serum uric acid and creatinine. The alteration in these kidney function biomarkers was more evidence in rats exposed to ZnO-NPs compared to its bulk counterpart. Our data are similar to other previous study indicated impairment of kidney functions with severe inflammatory response in animals exposed to ZnO-NPs (Fadda et al., 2012).

Administration of combination of B vitamins simultaneously with either ZnO-bulk or its NPs ingestion, greatly alleviated ZnO induced nephrotoxicity. This beneficial impact obtained by the used vitamins may attribute to their abilities to protect and stabilize cellular membranes by manipulating the ZnO toxicity. The anti-toxic and protective effects of B vitamins was previously documented (Wheatley, 2006 ;Mehta et al., 2008, 2011).

Previous study demonstrated that promotion of inflammatory cytokines expression by ZnO-NPs is considered as mediators of its reotoxicity (Fadda et al., 2012). This finding is coped with the present result which revealed elevation in the levels of immunological pro-inflammatory biomarkers including, TNF- α , and CRP, in serum of rats intoxicated with either ZnO- bulk or its NPs in relation to normal group.

TNF- α and CRP are two immuno-inflammatory injurious mediators increased in response to inflammation induced by metal oxide toxicity including ZnO (Sayes et al., 2007, Fadda et al., 2012, 2013). The up-regulation of TNF- α has a principle role in the activation of proinflammatory pathways in various cell types (D'Alessandris et al., 2007). It stimulate the production of other inflammatory cytokines including IL-6, the chief stimulator of CRP

production, leading to inflammatory tissue injury (Kerner et al., 2005).

Thus, attenuating inflammatory mediators production using anti-inflammatory drugs may protect against tissue injury and remote organ dysfunction

Oral ingestion of B vitamins along with either ZnO- bulk or its NPs administration presented in this study, markedly reduced the inflammatory biomarkers indicating their anti-inflammatory and immunomodulatory potential impacts. The anti-inflammatory of the used vitamins (B₃, B₆ and B₁₂) was previously reported (Miller, 2002; Biedron et al., 2008). Previous investigation demonstrated that vitamin B3 has the ability to reduce the elevated pro-inflammatory cytokines including TNF- α , IL-6 induced by LPS in human placenta. (Lappas and Permezel, 2011). Also the immunomodulatory and anti-inflammatory beneficial effects of vitamin B12 in treating many inflammatory diseases were documented (Miller, 2002; Scalabrino et al., 2008). Vitamin B₁₂ therapy could normalize TNF- α and epidermal growth factor levels in vitamin B₁₂ deficient patients (Scalabrino et al., 2008).

The current study showed also renal injury related to ZnO- bulk or its NPs toxicity stimulate the e expression of VEGF in serum of ZnO intoxicated rats versus normal ones. Clinical studies demonstrated that renal injury was found associated with renal peritubular capillaries (PTCs) losses which are essential to maintain the normal structure and function of renal tubules. The integrity of PTCs seems to be regulated by growth factors. Loss of PTCs may result in ischemia and these induce VEGF expression which is a potent regulator of angiogenesis, vascular survival, and vascular permeability (Choi et al., 2000). Expression of various tissue factors, cytokines, and chemokines in response to inflammatory tissue injury stimulate VEGF synthesizing cells such as platelets, immune cells, and inflammatory cells (Frank et al., 1999, Tonnesen et al., 2000). It was found that stimulation of angiogenesis may contribute to the transition from acute to chronic inflammation. Recent studies revealed that expression of this factor may

increase the permeability of newly generated vessels. High permeability may decrease the functionality of these neovessels and may in turn facilitate renal injury in chronic renovascular disease by allowing the leakage of injurious cytokines to the extra-vascular space. Beside, VEGF has been shown to promote tumor growth and it has been suggested to play a role in promoting atherosclerosis (Chade and Kelsen 2010; Iliescu et al., 2010). Additionally, some investigations demonstrated that TNF- α and VEGF expressions were significantly linked. Both TNF- α and VEGF may promote a procoagulant state, by increasing expression of tissue factor on endothelial cells and/or monocytes (Clauss et al., 1996, Mechtcheriakova et al., 2001). Increased tissue factor expression is thought to play a significant role in the development of multi-organ system failure in acute injury (Mechtcheriakova et al., 2001). This suggests the possibility that TNF- α and VEGF might act synergistically to potentiate renal injury and/or systemic organ dysfunction (Gurkan et al., 2003).

Co- ingestion of the used B vitamins combination, significantly reduced the dramatic increase in this angiogenic biomarker in sera of ZnO intoxicated rats, implying its anti-angiogenic potential action. Choi et al., (2011) revealed that vitamin B₃ derivative inhibits VEGF-mediated angiogenesis signaling in human endothelial cells (Choi et al., 2011). N-phenyl nicotinamides is a potent inhibitor of VEGF receptors (Dominguez et al., 2007). Also, vitamin B₆ mediated suppression of colon angiogenesis was previously reviewed (Matsubara et al., 2003). Furthermore, clinical study stated that chronic vitamin B₁₂ deficiency promoting the angiogenesis in a young vegetarian woman, which was reversible after treatment with B₁₂ (Aroni et al., 2008).

The damaging effect of ZnO on DNA has been previously studied (Fadda et al., 2012, 2013). The comet assay is a sensitive and a simple assay for detecting DNA damage at the level of individual cells (Singh et al., 1988).

Cell death or apoptosis is associated with DNA fragmentation (Tice and Strauss, 1995). With an increasing number of breaks, DNA pieces migrate freely into the tail of the comet, and in extreme cases (the apoptotic cell), the head and the tail are well separated. Tail length, percentage of total DNA in the tail and tail -DNA moment, reflect DNA damage, as it is directly related to the frequency of breaks over a wide range of damage (ColLins et al., 1995).

By using comet assay to detect DNA damage, the result indicated that either ZnO-bulk or its NPs intoxication induced kidney DNA damage as shown by significant increase in the tail length, DNA % in the tail and tail -DNA moment in kidney of

intoxicated rats compared with normal animals. The present data also showed that toxicity of ZnO induced oxidative stress in rat kidneys as observed by increased MDA (index of lipid peroxidation) coupled with a decrease in antioxidant enzyme, GPX, compared to normal animals. This effect was severe in rat kidneys intoxicated with ZnO- NPs. Previous studies suggested that ZnO induced DNA damage may be related to lipid peroxidation and oxidative stress (Xiong et al., 2011). ROS are known to react with DNA molecules, causing damage to both purine and pyrimidine bases as well as the DNA backbone (Martinez et al., 2003). In addition, MDA, a major product of lipid peroxidation, is a mutagenic and carcinogenic compound. This compound reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine, and deoxycytidine (Marnett, 2002; Niedernhofer et al., 2003). DNA damage resulting from any of these probable mechanisms may stimulate signal transduction pathways and cause interferences with normal cellular processes, thereby causing cell death or apoptosis (Sharma et al., 2009).

Co-administration of combination of B vitamins to rat groups intoxicated with either ZnO-bulk or its NPs effectively protected their kidney tissues from DNA damage and attenuated the increase in MDA as well as the decrease in the antioxidant enzyme, GPx. This result may indicate the antioxidant beneficial ability of B vitamins to alleviate the oxidative stress induced kidney DNA damage (Kannan and Jain, 2004; Moreira et al., 2009, Lappas and Permezel, 2011). Lappas and Permezel, (2011) reported that vitamin B₃ ingestion mitigated oxidative stress, and increased gene expression of antioxidant enzymes including GPx in LPS- induced toxicity. Also, in vitro study showed that vitamin B₃ has an important role in genomic stability, repairing of DNA damage and protecting against cytotoxic effects of DNA-damaging agents (Jacobson et al. 1999). Also Jia et al., (2008) suggested that nutritional supplementation of vitamin B₃ at high doses decreases oxidative stress induced DNA damage in experimental models. B₆ vitamin was also effective in protecting against lipid peroxidation, protein oxidation, and DNA damage (Mehta et al., 2011). Vitamin B₆ has a potential role in reducing oxidative stress markers associated with homocysteinemia (Mahfouz and Kummerow, 2004) or in preventing ROS formation and lipid peroxidation in a cellular model (Kannan and Jain, 2004). In addition, previous published data revealed that pretreatment of cultured lymphocytes with vitamin B₁₂ protected them from, oxidative DNA damage caused by pioglitazone (Alzoubi et al., 2012).

Apoptosis represents a key event after tissue injury and oxidative DNA damage (Sharma et al., 2009). The data obtained in the present study revealed

pronounced increased in the activity of biomarker of apoptosis, caspase 3, in kidneys of rats intoxicated with either ZnO- bulk or its NPs, suggesting that apoptosis might contribute to this metal oxide-induced DNA damage.

Intake of B vitamins combination to ZnO-intoxicated rats effectively down-modulated the increase in caspase 3 in their kidneys compared to normal animals. This result may indicate that the used vitamin B combination mediated protection against ZnO induced kidney tissue damage through its strong anti-apoptotic effect. The mechanism of their antiapoptotic effect may be related to their ability to inhibit oxidative DNA damage induced by ZnO. Vitamin B3 was found to inhibit alkylating agent-induced apoptotic neuro-degeneration in the developing rat brain (Ullah et al., 2011). Also, Endo et al., (2007) stated that vitamin B₆ suppressed apoptosis of NM-1 bovine endothelial cells induced by homocysteine and copper through its antioxidant effect. Vitamin B₁₂ was reported to have the ability to reverse dexamethasone-induced apoptosis in mesenchymal cell of mice during key periods of palatogenesis (He et al., 2010).

Conclusion:

The findings of the current study may suggest that prophylactic treatment with the tested combination of B vitamins Was beneficial in attenuating inflammation and apoptotic oxidative DNA damage induced in rat kidneys in response to the toxic effects of either ZnO- bulk or its NPs.

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