Prophylactic role of B vitamins against bulk and zinc oxide nano-particles toxicity induced oxidative DNA damage and apoptosis in rat livers

Jehad Mustafa Yousef and Azza Mostafa Mohamed
Biochemistry Department, King Abdulaziz University, Faculty of Science for Girl's, Jeddah, Saudi Arabia

Abstract: The aim of this work is to explore the protective of B vitamins (B_3, B_6 and B_{12}) against the hepatotoxic potency of either bulk zinc oxide (ZnO-bulk) or its nanoparticles (ZnO-NPs)-induced liver damage in rats. ZnO- bulk or its NPs were administered orally (500 mg/kg b.w.) for 10 successive days. The results revealed that oral co-administration of combination of B vitamins (250 mg B_3, 60 mg B_6 and 0.6 mg B_{12} /Kg body weight) daily for 3 weeks to rats intoxicated by either ZnO- bulk or its NPs markedly ameliorated increases in serum of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehdrogenase (LDH). The B vitamins also down-regulated increases in serum glucose level as well as increases in immuno-inflammatory biomarkers, including tumor necrosis factor-α (TNF-α) and C-reactive protein compared with intoxicated, untreated rats. Beside, the used agent successfully modulated the alterations in serum vascular endothelial growth factor (VEGF), attenuated liver oxidative DNA damage compared with ZnO intoxicated groups. We showed that the used B complex mitigated increased malondialdehyde (MDA), decrease in glutathione peroxidase (GPx) and increase in the apoptosis marker caspase 3 of liver tissue in response to either ZnO-bulk or its NP toxicity. In conclusion, early treatment with vitamin B complex may protect liver tissue from deleterious damage induced by the toxic effects of ZnO- bulk or its NPs.

Keywords: vitamin B complex, zinc oxide, deoxyribonucleic acid, tumor necrosis factor-α

INTRODUCTION

Nanoparticles (NPs), in comparison with bulk materials, have unique and novel characteristics and thus provide great chances for development of new industrial applications (Borm *et al.,* 2006). Many NPs are already used in manufacture or have the capacity to be used widespread in a range of applications (Nohynek *et al.,* 2007).

Manufactured NPs are inevitably released and present in the environment during manufacturing, transport, use, and disposal operations, suggesting that a fundamental understanding of their mode and range of toxicity is needed (Handy *et al.,* 2008; Lin *et al.,* 2010). Metal oxide NPs are manufactured in large scale for both industrial and household use. Some authors reported increasing application of these NPs indifferent commercial products, leading to environmental fate and potential toxicity (Kahru *et al.,* 2008).

The inflammation-driven effects are the prominent pathological mechanisms induced by NPs toxicity, including tissue fibrosis, oxidative tissue damage, and DNA damage. This making inflammation as a target for toxicological testing (Borm *et al.,* 2006; Lu *et al.,* 2009). Inflammation is a complicated set of responses that is dangerous when induced chronically by environmental pollutant such as inhaled particles (Donaldson *et al.,* 2006). The type, damages and outcome of inflammation differ, depending on the nature of the toxicant initiating the inflammation; the affected organ; the nature of the cellular exudates; its chronicity, seriousness and ability to resolve; and the genetic susceptibility of the individual

Zinc Oxide nanoparticles (ZnO-NPs) are typical metal oxide NPs and they are noncombustible and odorless white powders. They are produced abundantly and widely applied in a range of products including sunscreens, cosmetics, paint, paper, plastics and building materials (EPA 2007; Wang 2004). Previous study suggests that ZnO-NPs was bio-safe and biocompatible and could be used in biomedical materials (Berube, 2008). However, toxicological studies proved that ZnO-NPs had deleterious effects on human health and environmental animal species (Elder *et al.*, 2006, Xiong *et al.,* 2011). The bio-safety of ZnO-NPs is still a controversial question. ZnO-NPs was considered as a respiratory toxicant which leadsto metal fume fever (myalgia, cough, fatigue, etc.) (Beckett *et al.,* 2005). Recently, *in vivo* experimental studies showed that exposure to ZnO-NPs resulted in oxidative stress and inflammatory damaging effect in vascular/lung endothelial cells (Lin *et al.,*2009) as well as apoptosis in renal tissue (Fadda *et al.,* 2012). Animal experiments also demonstrated that most organs including liver, heart, kidney, pancreas, spleen and bone were target of oral exposure to 20- and 120-nm ZnO (Fadda *et al.,* 2012). Compared with the conventional toxicology, nano-particle materials are theoretically expected to be more toxic than their bulk ones because of

**Corresponding author:* e-mail: azzamohamed99@yahoo.com.

their greater surface reactivity and their capacity to penetrate into and accumulate within cells and organisms (Ispas *et al.,* 2009; Mironava *et al.,* 2010).

Recently, the effects of B vitamins that interfere directly with inflammatory response and oxidative damage have been described (Chen *et al.,* 2008, Lappas and Permezel, 2011).

Nicotinamide, also known as niacin (vitamin B_3), is found in nuts, dairy products, lean meats, fish, eggs, legumes and cereals. Beside its nutritional roles, vitamin B_3 was reported to have potential pharmacological activities. It has an important role in energy production via its major metabolite NAD^{++} (nicotinamide adenine dinucleotide (Maiese *et al.,* 2009). It has also anti-inflammatory, antioxidant (Biedron *et al.,* 2008, Lappas and Permezel, 2011), hepatoprotective (Chen *et al.,* 2008) and antiulcer (Abdallah 2010) properties. Previous study also revealed that nicotinamide administration showed a marked decrease of lipo-polysaccharides (LPS) induced gene expression and release of the immuno-inflammatory mediators including TNF-α, IL-6 and the chemokine, IL-8. Additionally, nicotinamide administration resulted in amelioration of LPS-induced oxidative stress, and increasing gene expression of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Lappas and Permezel 2011).

Vitamin B6, (namely pyridoxine, pyridoxamine or pyridoxal) and their phosphorylated derivatives, is an essential co-enzyme for numerous enzymatic reactions. It acts as a cofactor for enzymes involved in transamination, deamination decarboxylation, racemization and trans-sulfuration reactions (Depeint *et al.,* 2006). It used as a therapeutic agent in the treatment of cardiovascular disease (Wierzbicki, 2007), diabetes (Jain, 2007) and epilepsy (Gaby, 2007). The antioxidant and radical scavenging activities of the B_6vitamin have been previously documented. It has a strong role in attenuating oxidative stress biomarkers related to homocysteinemia (Mahfouz and Kummerow, 2004) or in preventing reactive oxygen species (ROS) formation and lipid peroxidation in a cellular model (Kannan and Jain, 2004).

Cobalamins (Cbl; vitamin B_{12} derivatives) are micronutrients used as a co-factor for the synthesis of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), the respective cofactors for cytosolic methionine synthase (MS) and mitochondrial L-methylmalonyl-CoA mutase (Solomon, 2007). It has fundamental therapeutic roles in the treatment of different pathological conditions. Cbl ingestion is potential in treating many inflammatory diseases, prophylaxis against oxidative stress-associated pathologies (Miller, 2002; Wheatley, 2006) and modulating the immune response (Scalabrino *et al.,* 2008). Cbl therapy ameliorates 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 $O_2^{\bullet-}$ production exceed the ability of superoxidedismutase (SOD) protection system (Moreira *et al.,* 2011). The reduced form of Cbl, cob (II) can scavenge $O_2^{\bullet-}$which is a significant mechanism by which Cbl can protect cells against oxidative damage (Moreira *et al.,* 2009). Vitamin supplements containing cyanocobalamin (CNCbl, vitamin B_{12}) reduced low-density lipoprotein oxidation in both patients and healthy persons as well as individuals with coronary artery disease (Earnest *et al.,* 2003) Clinical study illustrated that high doses of Cbl have been used to treat pernicious anemia for several years with no apparent toxicity (Mangiarotti *et al.,* 1986).

The current study was undertaken to study the prophylactic beneficial action of vitamin B complex (vitamins B_3, B_6 and B_{12}) against inflammation, oxidative DNA damage and apoptosis induced by toxicity of either ZnO-bulk or its NPs in rat livers

MATERIALS AND METHODS

Chemicals

ZnO-bulk and its NPs (<100 nm) powders were purchased from Sigma Co. (USA). Vitamin B_3, B_6 and B_{12}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 experimental design

Fifty healthy male albino rats (120-150g) of Sprague-Dawley strain were obtained from 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 housed in clean cages and maintained under standard conditions (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 diet with free access to tap water *ad libitum* for 1 week for acclimatization. After 1 week of acclimation, the animals were 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 vitamin B complex.
G5: ZnO-NPs intoxicated rats with co-administration of vitamin B complex.

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 B_3 (250 mg/Kg body weight, Godin *et al.,* 2012), B6 (60 mg/Kg body weight, Macêdo *et al.,* 2011) and B_{12} (0.6 mg/Kg body weight, Macêdo *et al.,* 2011) were administered orally in combination daily for three weeks. Three weeks later, therats of all groups were kept fasting over night (12-14 h), the blood samples were collected from each animal in different experimental 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 liver samples were collected, finely chopped and homogenized in ice-cold bid stilled water to yield 10% homogenates. The homogenates were centrifuged for 15 minutes at 10000g. at 4°C and the supernatants were used for estimation of some biochemical parameters.

Biochemical serum assay

Alanine aminotransferase (ALT) and Aspartate amino transferase (AST) activities were determined according to the method described by Bergmeyer *et al.,* (1985). Lactate dehydrogenase (LDH) activity was evaluated according to Bergmeyer (1975). 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 estimated 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 concentration of CRP present in the sample. The level of vascular endothelial growth factor (VEGF) was assayed by quantitative colorimetric sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems, UK) at 492 nm 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 liver tissue

Lipid peroxidation wasestimated by measuring the formed malondealdehyde (MDA) (index of lipid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). In this assay ared adduct was formed 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

Glutathione peroxidase (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 5,59-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

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

RESULTS

Serum liver damage markers, namely ALT, AST and LDH in the normal and different experimental rat groups intoxicated with either repeated doses of ZnO-bulk or its NPs are shown in table1. The two toxic forms of ZnO(G2 and G3 respectively) induced pronounced increases in these biomarkers compared with normal animals (G1), however the deviation in these biomarkers was sever in rats intoxicated with ZnO-NPs (G3). The intake of vitamin B complex to ZnO-bulk or its NPs (G4 and G5 respectively) significantly down-modulated the deterioration in these markers in relation to intoxicated either rat group.

The level of serum glucose in normal and ZnO-bulk intoxicated different rat groups is depicted in table1. The result showed marked increases in serum glucose level in rats intoxicated with either ZnO form, which was pronounced in rats ingested ZnO-NPs.

Table 1: Effect of vitamin B complex on the levels of serum liver function biomarkers in normal and ZnO-intoxicated rats

Parameters	Normal	ZnO-bulk	ZnO-NPs	ZnO-bulk+Vit B- complex	ZnO-NPs+Vit B-complex
ALT (U/L)	13,3±1.5	I 72.04± 2.6[a]	l39.34±1.2[a$]	41.73±1.5[a*]	46.5±1.7[a#]
AST (UKL)	32.1±1.8	97.36 ±7.2[a]	136.22±3.1[a$]	53.1±3.1[a*]	95.8±i4.05[a#]
LDH (U/L)	1339.26±51.25	1904.96±53.11[a]	2260.0±20.2[a$]	1516.8±757.05l[a*]	1943.3±40.4[a#]
Glucose mg/dl	82.66±1.53	96.3±2.5[a]	129.06±3.5[a$]	85.6±2.05[a*]	l06.6±2.1[a#]

Data are presented as mean ± SD of 6 rats, [a] $P \leq 0.001$, [b] $P \leq 0.01$,[n] Non significant compared with normal group, [*] $P \leq 0.01$, [$] $P \leq 0.001$ 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

Table 2: Effect of vitamin B complex on the levels of serum inflammatory biomarkers in normal and ZnO- intoxicated rats

Parameters	Normal	ZnO-bulk	ZnO-NPs	ZnO-bulk+Vit B- complex	ZnO-NPs+Vit B-complex
TNF-a (pg/ml)	9,78 ±1.4	33.65±1.5 [a]	38.4±.2.3[a$]	22.6±2.3[a*]	24.8 ±1.6[a#]
CRP (ng/ml)	2.6±l.1	16.47±0.8 [a]	19.4±1.4[a$]	10.38±1.02[a*]	8.5±l.12[a#]
VEGF (pgfml)	l74.4±4.7	204.6±3.68 [a]	2l7.35±7.5[a$]	l26.1±5.3[a*]	l36.6±2.9[a#]

Data are presented as mean±□S.D. from 6 rats, [a]P□≤□0.001, [b]P≤.01, [c]P≤0.05 compared with the normal group, [*]P≤□0.001, [$]$P$□≤0.001 , [$$]P□≤0.01, [$$$]P□≤0.05 compared with ZnO-bulk intoxicated group, [#]P≤□0.001, compared with ZnO-NPs intoxicated group using ANOVA followed by Bonferroni as a post-ANOVA test

Table 3: Effect of vitamin B complex on some liver tissue biomarkers in ZnO different experimental rat groups

Parameters	Normal	ZnO-bulk	ZnO-NPs	ZnO-bulk+Vit B- complex	ZnO-NPs+Vit B-complex
MDH (nmol/g)	12.l3±0,58	16.7±0.75[a]	19.8±0.9[a$]	13.1±0.61[a*]	13.4±0.23[a#]
GPX (nmol"min/mgprotcin)	26.65±1.12	20.l±0.53[a]	18,03±0.47[a$]	24.16±0.55[a*]	24.46±0.51[a#]
Caspase-3	2l7.8±2.4	325.76±5.05[a]	370.8±2,6[a$]	255,6±4.06[a*]	295.26±5.05[a#]
Tail-DNA length (um)	2.8±0.2	5.47±0.23[a]	6.O2±0.07[a$]	3.47±0.075[a*]	4,2±10.10[a#]
Tail-DNA%	3.2±0.01	4.6±0.10[a]	4,9±0.09[a$]	3.1±0.11[a*]	3.7±0.25[a#]
Uni! Tail –DNA moment	10.33±0.6	25.09±0.7[a]	28.7±1.05[a$]	l3.7±0.95[a*]	15.l±0.83[a#]

Data are presented as mean±□S.D. from 6 rats, [a]P≤0.001, [b]P≤0.01, [c]P≤0.05,[n] Non significant compared with the normal group[*]P≤0.001, [$$]P≤0.01, [$]$P$≤0.05 compared with ZnO-bulk intoxicated group, [#]P≤0.001,compared with ZnO-NPs intoxicated group using ANOVA followed by Bonferroni as a post-ANOVA test

The levels of some immunological pro-inflammatory biomarkers, including TNF-α, and CRP, in the normal and different experimental rat groups intoxicated with either form of ZnO-bulk are illustrated in table 2. These biomarkers were dramatically elevated in the serum of rats intoxicated with either form of ZnO compared with the normal group; however, the deviation in these biomarkers was more evident in ZnO-NPs rat group. 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 serum of rats intoxicated with either ZnO-bulk or its NPs compared with normal animals (table.2). Co-administration of vitamin B complex, markedly reduced the dramatic increase in this factor in sera of ZnO-intoxicated rats compared with either intoxicated, untreated animal group.

The effect of the administration of either ZnO-bulk or its NPs on liver DNA of rats is shown in table 3 and fig.1, respectively. A significant increase in the tail length, DNA % (tail DNA content) and tail-DNA moment was shown in the liver tissues of rats intoxicated with either ZnO- bulk or its NPs. This effect was pronounced in ratlivers intoxicated with the repeated doses of ZnO-NPs. Co-administration of the current agent, in ZnO-intoxicated rats significantly protected their livers from DNA damage as indicated by a decrease in tail length, DNA % and tail -DNA moment compared with intoxicated rats.

Table 3 shows the levels of MDA and GPx (antioxidant biomarker) in normal and ZnO intoxicated different rat groups. The data revealed that toxicity of this metal oxide induced increased MDA with concomitant decrease in an antioxidant enzyme, GPX, compared to normal animals. This effect was severe in rat livers ingested ZnO-NPs. Co-ingestion of vitamin B complex 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.

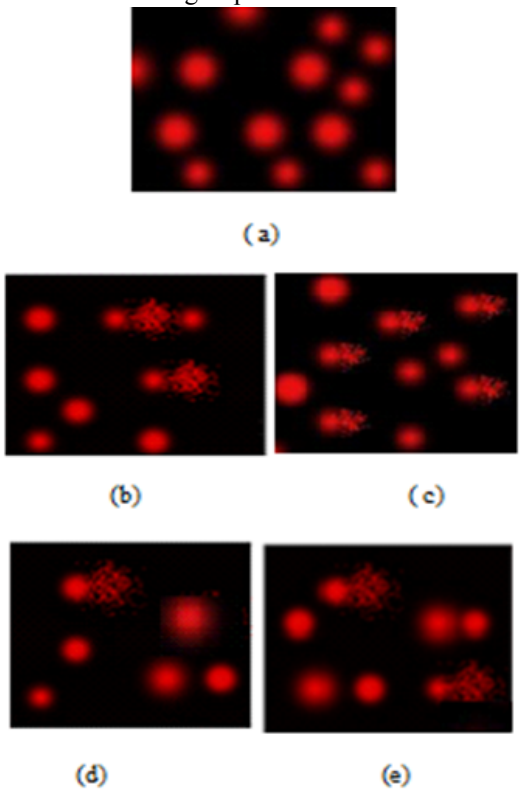

Fig. 1: COMET assay showing the degree of DNA damage in the liver tissue in intoxicated rats with either bulk or ZnO-NPs, and the effect of vitamin B complex treatment on the level of DNA damage. (a) normal control group, (b) group intoxicated with bulk ZnO (c), group intoxicated with ZnO-NPs, (d) group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

Table 3 shows that the liver apoptosis biomarker caspase 3 was significantly up-regulated in rats administered either repeated doses of ZnO-bulk or its NPs. Co-administration of the studied agents to either ZnO-intoxicated rat group, beneficially down-modulated the increase in liver caspase 3.

DISCUSSION

Some studies have reported that the toxicity of bulk metal oxide and its NPs (d < 100 nm) were related to diseases of body vital organs including liver (Wang *et al.,* 2008, Xiong *et al.,* 2011). NPs are severely toxic than their bulk ones due to their greater surface reactivity and their capacity to penetrate into and accumulate within cells and organisms (Ispas *et al.,* 2009; Mironava *et al.,* 2010).

In consistent with previous investigations, the current study revealed that ingestion of repeated doses of either ZnO-bulk or its NPs (500mg/Kg for 10 consecutive days) to rats for 10 consecutive days, induced liver damage as documented by marked elevation of serum ALT, AST and LDH in both intoxicated rat groups, indicating cellular leakage and loss of the functional integrity of liver cell membranes. These changes were more severe in rats intoxicated with ZnO-NPs (Wang *et al.*,2008). It has demonstrated that NPs, including ZnO, may cause more inflammatory tissue damage than larger particles of the same material at the same mass dose delivery (Rahman *et al.,* 2002, Wang *et al.*,2008, and Xiong *et al.,* 2011).

Co-administration of vitamin B complex to either intoxicated rat groups significantly reduced the serum levels of liver function biomarkers compared with intoxicated rat groups. This may indicate that the used vitamins acts as effective hepatoprotective against ZnO caused liver dysfunction. The positive response obtained by the used vitamin B complex may attribute to their ability to protect and stabilize cellular membranes by manipulating the ZnO toxicity. The anti-toxic and the hepato-protective effects of B vitamins were previously reported (Wheatley, 2006, Chen *et al.,* 2008, Mehta *et al.,* 2008, 2011).

Some studies have demonstrated that pathogenic mechanisms initiated by bulk metal oxide and its NPs are dominated by inflammation-driven effects, including, oxidative stress, apoptosis and DNA damage (Borm *et al.,* 2006; Lu *et al.,* 2009; Xiong *et al.,* 2011).

In the present study, it was found that a marked increase in serum glucose level as well as in the levels of immunological pro-inflammatory biomarkers including TNF-α and CRP, in rat serum intoxicated with either ZnO-bulk or its NPs in relation to normal group, implying metabolic and immune disorder.

TNF-α is one of the most commonly inflammatory injurious chemokine immunological markers increased in response to different metal oxide toxicity including ZnO (Sayes *et al.,* 2007; Veranth *et al.,* 2007). While CRP is a member of the pentaxin protein family, synthesized by the liver in response to inflammation (Pepys and Baltz, 1983). 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 triggers the production of other inflammatory cytokines including IL-6, the chief stimulator of CRP production, leading to inflammatory tissue injury (Kerner *et al.,* 2005). On the other hand, it was reported that up-regulation of CRP is closely associated with metabolic disturbances including, insulin resistance and related complications such as fatty liver disease and hyperglycemia (Xi *et al.,* 2011).

Thus, a protective strategy attenuates production of inflammatory mediators could protect against tissue injury and remote organ dysfunction The intake of vitamin B

complex immediately with either ZnO-bulk or its NPs ingestion presented in this study, markedly attenuate the increases in serum glucose and inflammatory biomarkers. The anti-inflammatory of the used vitamins (B_3, B_6 and B_{12}) was previously documented (Miller, 2002; Biedron *et al.,* 2008). Some studies showed that nicotinamide (vit B_3) administration resulted in a marked reduction of pro-inflammatory cytokines including TNF-α, IL-6 induced by LPS in human placenta (Lappas and Permezel, 2011). VitaminB_{12} supplementation was also beneficial in modulating the immune response and treating many inflammatory diseases, (Miller, 2002; Scalabrino *et al.,* 2008). Vitamin B_{12} therapy normalized levels of TNF-α and epidermal growth factor in vitamin B_{12}-deficient patients (Scalabrino *et al.,* 2008).

Liver injury leads to vascular deterioration and local tissue hypoxia starting early in disease course. In this case, hypoxia not only acts as an upsetting factor of cell damage and inflammation, but also as suppressor of liver regeneration, a major stimulus of angiogenesis (growth of new blood vessels from pre-existing vessels), fibrogenesis, and a promoter of liver carcinogenesis (Rosmorduc and Housset, 2010; Paternostro *et al.,* 2010). The present study demonstrated significant increase in serum VEGF (angiogenic factor) of rats intoxicated with either ZnO-bulk or its NPs compared with normal ones. This result is supported by previous study demonstrated the expression of various tissue factors, cytokines, and chemokines in response to inflammatory tissue injury (Verheul *et al.,* 2000; Lingen, 2001). Up-regulation of VEGF has been demonstrated to be a major contributor to angiogenesis that stimulates vasculogenesis (Ding *et al.,* 2004). Angiogenesis is a part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF has a principle role in the generation of new blood vessels after injury, to bypass blocked vessels (Prior *et al.,* 2004). However, it was found that stimulation of angiogenesis may lead to the transition from acute to chronic inflammation. Previous study demonstrated that neo-vessels can significantly contribute to perpetuation of the inflammatory response by expressing chemokines and adhesion molecules promoting the activation of inflammatory cells (Jackson *et al.,* 1997). Some authors illustrated that a positive correlation between the VEGF expression and progression of fibrogenesis (Rosmorduc and Housset, 2010). VEGF has been reported to be able to trigger hepatic stellate cells (HSCs) proliferation (Olaso *et al.,* 2003) increase deposition of fibrogenic proteins, extra cellular matrix protein (ECMP) components as collagen I (Corpechot *et al.,* 2002). In addition, Previous studies stated 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 production is thought to play the major cause 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 liver injury and/or systemic organ dysfunction (Gurkan *et al.,* 2003).

The use of anti-angiogenic agents may then represent an attractive alternative therapeutic tool to prevent or significantly slow down fibrosis progression towards cirrhosis, which also represents the main risk factor for liver cancer development.

The protective ingestion of the used vitamin B complex markedly reduced the dramatic increase in this angiogenic biomarker in serum of ZnO intoxicated rats, suggesting its anti-angiogenic beneficial action. Choi *et al.,* (2011) stated that nicotinamide (vit B_3) derivative inhibits VEGF-mediated angiogenesis signaling in human endothelial cells (Choi *et al.,* 2011). N-phenyl nicotinamides was potent anti-angiogenic through inhibiting VEGF receptors (Dominguez *et al.,* 2007). Also, vitamin B6 mediated suppression of colon angiogenesis was previously reviewed (Matsubara *et al.,* 2003). Furthermore, previous clinical study reported that chronic vitamin B_{12} deficiency promoting the angiogenesis in a young vegetarian woman, which was reversible after treatment with B_{12} (Aroni *et al.,* 2008).

The damaging effect of metaloxide on DNA has been shown in previous study (Gurr *et al.,* 2005). The comet assay is an accurate and a simple assay for evaluating DNA damage at the level of individual cells (Singh *et al.,* 1988).

Apoptotic cell death due to fragmentation lead to increased DNA migration (Tice and Strauss 1995). With an increasing number of breaks, DNA pieces migrate freely into the tail of the comet, and in 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 (ColLins *et al.,* 1996).

Use comet assay to detect DNA damage indicated that either ZnO-bulk or its NPs intoxication induced liver DNA damage documented by a significant increase in the tail length, DNA % in the tail and tail -DNA moment in livers of rats. The current result also showed that toxicity of this metal oxide induced oxidative stress in rat livers as showed by increased MDA (index of lipid peroxidation) with concomitant decrease in an antioxidant enzyme, GPx, compared to normal animals. This effect was severe in rat livers ingested ZnO- NPs. Previous studies suggested that ZnO induced DNA damage may be related to lipid peroxidation and oxidative stress (Xiong *et al.,* 2011). ROS react with DNA, causing damage 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 mechanisms may elicit signal transduction pathways leading to apoptosis or interfere with normal cellular processes, thereby causing cell death (Sharma *et al.,* 2009).

Co-administration of vitamin B complex to rat groups intoxicated with either ZnO-bulk or its NPs effectively protected their liver tissues from DNA damage and ameliorated the increase in lipid peroxidation as well as the decrease in the antioxidant enzyme, GPx. This result give a clue to the ability of the B vitamins to mitigate the oxidative stress induced liver DNA damage which may relate to their antioxidant effect (Kannan and Jain, 2004, Moreira *et al.,* 2011, Lappas and Permezel, 2011). This indication is supported by Lappas and Permezel (2011) who reported that nicotinamide administration was beneficial in attenuating LPS- induced oxidative stress, and stimulating gene expression of antioxidant enzymes including GPx. Also, *in vitro* study reported that nicotinamide has important role ingenomic stability, repairing of DNA damage and protecting against cytotoxic effects of DNA-damaging agents (Jacobson *et al.,* 1999). Jia *et al.* (2008) suggested that nutritional supplementation of nicotinamide at high doses decreases oxidative stress induced DNA damage in experimental models. B_6vitamin was also effective in protecting hepatocytes from iron-catalyzed lipid peroxidation, protein oxidation and DNA damage (Mehta *et al.,* 2009). Vitamin B_6 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 B_{12} protected them from, oxidative DNA damage caused by pioglitazone (Alzoubi *et al.,* 2012).

Apoptosis represents a key event after liver injury and oxidative DNA damage (Sharma *et al.,* 2009). The data generated in the current work showed markedly increased activity of the apoptosis biomarker caspase3 in liver tissue of rats intoxicated with either ZnO- bulk or its NPs, suggesting that apoptosis might contribute to this metal oxide-induced DNA damage.

Co-administration of the studied B vitamins to ZnO-intoxicated rats beneficially down-modulated the increase in liver caspase 3. This result may indicate that the used vitamin B combination mediated protection against ZnO induced liver tissue damage through its strong anti-apoptotic effect. The mechanism of their anti-apoptotic effect may be related to their ability to inhibit oxidative DNA damage induced by ZnO. Nicotinamide was reported 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 B_6suppressed 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 counteract dexamethasone-induced apoptosis in mesenchymal cell of mice during key periods of palatogenesis (He *et al.,* 2010).

CONCLUSION

The findings of the current study suggest that prophylactic supplementation of vitamin B complex may be beneficial against inflammation and apoptotic oxidative DNA damage induced in rat livers by toxic effects of either ZnO- bulk or its NPs.

ACKNOWLEDGEMENTS

This paper was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No.(217/363/1432). The authors, therefore, acknowledge with thanks DSR technical and financial support.

REFERENCES

Abdallah DM (2010). Nicotinamide alleviates indomethacin-induced gastric ulcers: A novel anti-ulcer agent. *Eur. J. Pharmacol*., **627**: 276-280.

Alzoubi K, Khabour O, Hussain N, Al-Azzam S and Mhaidat N (2012). Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat. Res*., **748**: 48-51.

Aroni K, Anagnostopoulou K, Tsagroni E and Ioannidis E (2008). Skin hyperpigmentation and increased angiogenesis secondary to vitamin B12 deficiency in a young vegetarian woman. *Acta. Derm. Venereol*., **88**(2): 191-192.

Beckett WS, Chalupa DF, Pauly-Brown A, Speers DM, Stewart JC, Frampton MW, Utell MJ, Huang LS, Cox C, Zareba W and Oberdörster G (2005). Comparing inhaled ultra fine versus fine zinc oxide particles in healthy adults: A human inhalation study. *Am. J. Respir. Crit. Care. Med*., **171**(10): 1129-1135.

Bergmeyer HU (1975). Determination of lactate dehydrogenase. *J. Clin. Chem. Biochem*., **13**: 269.

Bergmeyer HV, Herder M and Rej R (1986). Approved recommendation (1985) on IFCC methods for the measurement of catalytical concentration of enzymes. Patr 2.IFCC method for aspartate aminotransferase. *J. Clin.Chem. Clin. Biochem*., **24**: 497.

Berube DM (2008). Rhetorical gamesmanship in the nano debates over sunscreens and nano particles. *J. Nanopart. Res*., **10**: 23-37.

Biedron R, Ciszek M, Tokarczyk M, Bobek M, Kurnyta M and Slominska EM (2008). 1-Methylnicotinamide and nicotinamide: Two related anti-inflammatory

agents that differentially affect the functions of activated macrophages. *Arch. Immunol. Ther. Exp*., **56**: 127-134

Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H and Donaldson K (2006). The potential risks of nanomaterials: A review carried out for ECETOC. *Part. Fibre. Toxicol*., **3**: 11

Buege JA and Aust SD (1978). Microsomal lipid peroxidation. *Methods. Enzymol*., **52**: 302-315.

Chen YH, Wang MF, Liao JW, Chang SP and Hu ML (2008). Beneficial effects of nicotinamide on alcohol-induced liver injury in senescence-accelerated mice. *Biofactors*., **34**: 97-107.

Choi HE, Yoo MS, Choi JH, Lee JY, Kim JH, Kim JH, Lee JK, Kim GI, Park Y, Chi YH, Paik SH, Lee JH and Lee KT (2011). BRN-103, a novel nicotinamide derivative, inhibits VEGF-induced angiogenesis and proliferation in human umbilical vein endothelial cells. *Bioorg. Med. Chem. Lett*., **21**(21): 6236-6241.

Clauss M, Grell M, Fangmann C, Fiers W, Scheurich P, and Risau W (1996). Synergistic induction of endothelial tissue factor by tumor necrosis factor and vascular endothelial growth factor: Functional analysis of the tumor necrosis factor receptors. *FEBS Lett*., **390**: 334-338.

ColLins AR, Dusinska M, Gedik CM and Stetina R (1996). Oxidative damage to DNA: Do we have a reLiabLe biomarker? Environ. *Health. Perspect*., **104**: 465-469.

Corpechot C, Barbu V, Wendum D, Kinnman N, Rey C, Poupon R, Housset C and Rosmorduc O (2002). Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. *Hepatol*., **35**: 1010-1021.

D'Alessandris C, Lauro R, Presta I and Sesti G (2007). C-reactive protein induces phosphorylation of insulin receptor substrate-1 on Ser307 and Ser 612 in L6 myocytes, thereby impairing the insulin signalling pathway that promotes glucose transport. Diabetologia, **50**: 840-849.

Depeint F, Bruce WR, Shangari N, Mehta R and O'Brien, PJ (2006). Mitochondrial function and toxicity: Role of B vitamins on the one-carbon transfer pathways. *Chem. Biol. Interact*, **163**: 113-132.

Ding YH, Luan XD and Li J (2004). Exercise-induced over expression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. *Curr. Neurovasc. Res*., **1**(5): 411-420.

Dominguez C, Smith L, Huang Q, Yuan C, Ouyang X, Cai L, Chen P, Kim J, Harvey T, Syed R, Kim TS, Tasker A, Wang L, Zhang M, Coxon A, Bready J, Starnes C, Chen D, Gan Y, Neervannan S, Kumar G, Polverino A and Kendall R (2007). Discovery of N-phenyl nicotinamides as potent inhibitors of Kdr. *Bioorg. Med. Chem. Lett*., **17**(21): 6003-6008.

Donaldson K, Aitken R, Tran L, Stone V, Duffin R and Forrest G (2006). Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol. Sci*., **92**(1): 5-22.

Earnest CP, Wood KA and Church TS (2003). Complex multivitamin supplementation improves homocysteine and resistance to LDL-C oxidation. *J. Am. Coll. Nutr*., **22**: 400-407.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L and Carter J (2006). Translocation of inhaled ultra fine manganese oxide particles to the central nervous system. *Environ. Health. Perspect*., **114**: 1172–1178.

Endo N, Nishiyama K, Okabe M, Matsumoto M, Kanouchi H and Oka T (2007). Vitamin B6 suppresses apoptosis of NM-1 bovine endothelial cells induced by homocysteine and copper. *Biochim. Biophys. Acta*., **1770**: 571-577.

Environmental Protection Agency (2007). Science Policy Council, Nanotechnology Workgroup Nanotechnology White Paper.

Fadda LM, Abdel Baky NA, Al-Rasheed NM, Al-rasheed NM, Fatani AJ and Atteya M (2012). Role of quercetin and arginine in ameliorating nano zinc oxide-induced nephrotoxicity in rats. *BMC. Complement. Altern. Med*., **12**: 60.

Gaby AR (2007). Natural approaches to epilepsy. *Altern. Med. Rev*., **12**: 9-24.

Godin AM, Ferreira WC, Rocha LT, Ferreira RG, Paiva AL, Merlo LA, Nascimento EB Jr, Bastos LF and Coelho MM (2012). Nicotinic acid induces anti-nociceptive and anti-inflammatory effects in different experimental models. *Pharmacol. Biochem. Behav*. **101**(3): 493-498.

Gurkan OU, O'Donnell C, Brower R, Ruckdeschel E and Becker PM (2003). Differential effects of mechanical ventilatory strategy on lung injury and systemic organ inflammation in mice. *Am. J. Physiol. Lung. Cell. Mol. Physiol*., **285**: L710-L718.

Gurr JR, Wang AS, Chen CH and Jan KY (2005). Ultra fine titanium dioxide particles in the absence of phopactivation can induce oxidative DNA damage to human bronchial epithelial cells. *Toxicol*., **15**: 66-73.

Handy RD, Kammer FVD, Lead JR, Hasselloev MO, Wen R and Crane M (2008). The ecotoxicity and chemistry of manufactured nanoparticles. *Ecotoxicol*., **17**: 287-314.

He W, Meng T, Lu SJ, Zheng Q, Li CH, Wu M and Shi B (2010). Vitamin B12 counteracts dexamethasone-induced proliferation and apoptosis during key periods of palatogenesis in mice. *Ann. Plast. Surg*., **64**(4): 466-470.

Ispas C, Andreescu D, Patel A, Goia DV, Andreescu S and Wallace KN (2009). Toxicity and developmental defects of different sizes and shape nickel nano particles in Zebra fish. *Environ. Sci. Technol*., **43**: 6349-6356.

Jackson JR, Seed MP, Kircher CH, Willoughby DA and Winkler JD (1997). The codependence of angiogenesis and chronic inflammation. *FASEB. J*., **11**: 457-465.

Jacobson EL, Shieh WM and Huang AC (1999). Mapping the role of NAD metabolism in prevention and treatment of carcinogenesis, *Mol. Cell. Biochem.,* **193**: 69-74.

Jain SK (2007). Vitamin B6 (pyridoxamine) supplementation and complications of diabetes. *Metabolism*, **56**: 168-171.

Jia H, Li X, Gao H, Feng Z, Li X, Zhao L, Jia X, Zhang H and Liu J (2008). High doses of nicotinamide prevent oxidative mitochondrial dysfunction in a cellular model and improve motor deficit in a Drosophila model of Parkinson's disease. *J. Neurosci. Res.*, **86**(9): 2083-2090.

Kahru A, Dubourguier HC, Blinova I, Ivask A and Kasemets K (2008). Biotests and biosensors for ecotoxicology of metal oxide nano particles: A minireview. *Sensors,* **8**: 5153-5170.

Kannan K and Jain SK (2004). Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H2O2-treated U937 monocytes. *Free. Radic. Biol. Med*., **36**: 423-428.

Kerner A, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, Yishai L, Brook G J and Aronson D (2005). Association between elevated liver enzymes and C-reactive protein possible hepatic contribution to systemic inflammation in the metabolic syndrome. *Arterioscler. Thromb. Vasc. Biol*., **25**: 93-197.

Lappas M and Permezel M (2011). The anti-inflammatory and antioxidative effects of nicotinamide, a vitamin B3 derivative are elicited by FoxO3 in human gestational tissues: implications for preterm birth. *J. Nutr. Biochem*., **22**: 1195-1201.

Lin DH, Tian XL, Wu FC and Xing BS (2010). Fate and transport of engineered nano-materials in the environment. *J. Environ. Qual*. **39**: 1896-1908.

Lin WS, Xu Y, Huang CC, Ma Y, Shannon KB, Chen DR and Huang YW (2009). Toxicity of nano- and micro-sized ZnO particles in human lung epithelial cells. *J. Nanopart. Res*., **11**: 25-29.

Lingen MW (2001). Role of leukocytes and endothelial cells in the development of angiogenesis in inflammation and wound healing. *Arch. Pathol. Lab. Med*., **125**: 67-71

Lu S, Duffin R, Poland C, Daly P, Murphy F and Drost E (2009). Efficacy of simple short-term *in vitro* assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation. *Environ. Health. Perspect*., **117**: 241-247.

Macêdo DS, Oliveira GV, Gomes PX, Araújo FY, Souza CM, Vasconcelos SM, Viana GS, Sousa FC and Carvalho AF (2011). B vitamins attenuate haloperidol-induced orofacial dyskinesia in rats: Possible involvement of anti-oxidant mechanisms. *Behav. Pharmacol*., **22**(7): 674-680

Mahfouz MM and Kummerow FA (2004). Vitamin C or Vitamin B6 supplementation prevent the oxidative stress and decrease of prostacyclin generation in homocysteinemic rats. *Int. J. Biochem. Cell Biol.,* **36**: 1919-1932.

Maiese K, Chong ZZ, Hou JL and Shang YC (2009). The vitamin nicotinamide: translating nutrition into clinical care. *Molecules*, **14**: 3446-3485.

Mangiarotti G, Canavese C, Salomone M, TheaA, Pacitti A, Gaido M, Calitri V, Pelizza D, Canavero W and Vercellone A (1986). Hypervitaminosis B12 in maintenance hemodialysis patients receiving massive supplementation of vitamin B12. *Int. J. Artif*. Organs, **9**: 417-420.

Marnett LJ (2002). Oxy radicals, lipid peroxidation and DNA damage. *Toxicol*., 181-182: 219-222.

Martinez GR, Loureiro AP, Marques SA, Miyamoto S, Yamaguchi LF, Onuk J, Almeida EA, Garcia CC, Barbosa LF, Medeiros MH and Di Mascio P (2003). Oxidative and alkylating damage in DNA. *Mutat. Res*., **544**: 115-127.

Matsubara K, Komatsu S, Oka T and Kato N(2003). Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). *J. Nutr. Biochem.*, **14**(5): 246-250.

Mechtcheriakova D, Schabbauer G, Lucerna M, Clauss M, De Martin R, Binder BR and Hofer E (2001). Specificity, diversity and convergence in VEGF and TNF-alpha signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. *FASEB. J*., **15**: 230-242.

Mehta R, Dedina L and O'Brien PJ (2011). Rescuing hepatocytes from iron-catalyzed oxidative stress using vitamins B1 and B6. *Toxicol. in vitro*., **25**: 1114-1122.

Mehta R, Shangari N and O'Brien PJ (2008). Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents. *Mol. Nutr. Food Res*., **52**: 379-385.

Mehta R, Wong L and O'Brien PJ (2009). Cytoprotective mechanisms of carbonyl scavenging drugs in isolated rat hepatocytes. *Chem. Biol. Interact*. **178**: 317-323.

Miller JW (2002). Vitamin B12 deficiency, tumor necrosis factor-alpha and epidermal growth factor: A novel function for vitamin B12? *Nutr. Rev.,* **60**: 142-144.

Mironava T, Hadjiargyrou M, Simon M, Jurukovski V and Rafailovich MH (2010). Gold nanoparticles cellular toxicity and recovery: Effect of size, concentration and exposure time. *Nanotoxicol*., **4**: 120-37.

Moreira ES, Brasch NE and Yun J (2011). Vitamin B12 protects against superoxide-induced cell injury in human aortic endothelial cells. *Free. Radical. Biol. Med*., **51**: 876-883.

Nath R, Raser KJ, Staeord D, Hajimohammadreza I, Posner A, Allen H, Talanian RV, Yuen PW, Gilbertsen RB and Wang KKW (1996). Nonerythroid alpha-spectrin breakdown by calpain and ICE-like protease(s) in apoptotic cells: Contributory roles of both protease families in neuronal apoptosis. *Biochem. J*., **319**: 683-690.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE and Marnett LJ (2003). Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J. Biol. Chem*., **278**: 31426-31433.

Nohynek GJ, Lademann J, Ribaud C and Roberts MS (2007). Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Crit. Rev. Toxicol*., **37**(3): 251-277.

Olaso E, Salado C, Egilegor E, Gutierrez V, Santisteban A, Sancho-Bru P, Friedman SL and Vidal-Vanaclocha F (2003). Proangiogenic role of tumor-activated hepatic stellate cells in experimental melanoma metastasis. *Hepatology*, **37**: 674-685.

Olive PL, Banath JP and Durand RE (1990). Heterogeneity in radiation induced DNA damage and repair in tumor and normal cells using the ‘‘Comet’’ assay. *Radiat. Res*., **122**: 86-94.

Paternostro C, David E, Novo E and Parola M (2010). Hypoxia, angiogenesis and liver fibrogenesis in the progression of chronic liver diseases *World. J. Gastroenterol*., **16**(3): 281-288.

Pepys MB and Baltz ML (1983). Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol*., **34**: 141-212.

Prior BM, Yang HT and Terjung RL (2004). "What makes vessels grow with exercise training? *J. Appl. Phys*., **97**(3): 1119-1128.

Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG and Schiffman D (2002). Evidence that ultra fine titanium dioxide induces micronuclei and apoptosis in syrian hamster embryo fibroblasts. *Environ. Health, Perspect*., **110**: 797-800.

Rosmorduc O and Housset C (2010). Hypoxia: A link between fibrogenesis, angiogenesis and carcinogenesis in liver disease. *Semin. Liver Dis*., **30**(3): 258-270.

Rotruck JT, Pope AL, Ganther HE, Swanson AB and Hafeman DG (1973) Selenium: Biochemical role as a component of glutathione peroxidase. *Science,* **179**: 588-590.

Sayes CM, Reed KL and Warheit DB (2007). Assessing toxicity of fine and nanoparticles: Comparing *in vitro* measurements to *in vivo* pulmonary toxicity profiles. *Toxicol. Sci.*. **97**: 163-180.

Scalabrino G, Veber D and Mutti E (2008). Experimental and clinical evidence of the role of cytokines and growth factors in the pathogenesis of acquired cobalamin-deficient leukoneuropathy. *Brain. Res. Rev*., **59**: 42-54.

Sharma V, Shukla RK, Saxena N, Parmar D, Das M and Dhawan A (2009). DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicol. Lett*., **185** : 211-218.

Singh NP, McCoy MT, Tice RR and Schneider EL (1988). A simple technique foe quantitation of low levels of DNA damage in individual cells. *Exp. Cell. Res*., **175**: 184-191.

Solomon LR (2007). Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in patho-physiology, diagnosis and treatment. *Blood Rev*., **21**: 113-130.

Tice RR and Strauss GHS (1995). The single cell gel e[ectrophoresis comet assay - a potential tool for detecting radiation-induced DNA damage in humans. *Stem. CelLs*., **13**(Suppl 1): 207-214.

Ullah N, Lee HY, Naseer MI, Ullah I, Suh JW and Kim MO (2011). Nicotinamide inhibits alkylating agent-induced apoptotic neurodegeneration in the developing rat brain. *PLoS. One*. **6**(12): 27093

Veranth JM , Kaser EG , Veranth MM, Koch M and Yost GS (2007). Cytokine responses of human lung cells (BEAS-2B) treated with micron-sized and nanoparticles of metal oxides compared to soil dusts. *Particle and Fibre. Toxicol*., **4**: 2-19.

Verheul HM, Jorna AS, Hoekman K, Broxterman HJ, Gebbink MF and Pinedo HM (2000). Vascular endothelial growth factor-stimulated endothelial cells promote adhesion and activation of platelets. *Blood*, **96**: 4216-4221.

Wang B, Feng WY, Wang M, Wang TC, Gu YQ, Zhu MT, Ouyang H, Shi JW, Zhang F, Zhao YL, Chai ZF, Wang HF and Wang J (2008). Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice. *J. Nanopart. Res*., **10**(2): 263-276.

Wang ZL (2004). Zinc oxide nanostructures: Growth, properties and applications. *J. Phys. Condens. Matter*, **16**: 829-858.

Wheatley CA (2006). Scarlet pimpernel for the resolution of inflammation? The role of supra-therapeutic doses of cobalamin, in the treatment of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic or traumatic shock. *Med. Hypotheses*., **67**: 124-142.

Wierzbicki AS (2007). Homocysteine and cardiovascular disease: a review of the evidence. *Diab. Vasc. Dis. Res*., **4**: 143-150.

Xi L, Xiao C, Bandsma RHJ, Naples M, Adeli K and Lewis GF (2011). C-Reactive protein impairs hepatic insulin sensitivity and insulin signaling in rats: Role of mitogen- activated protein Kinases. *Hepatol*., **53**: 127-135.

Xiong D, Fang T, Yu L, Sima X and Zhu W (2011). Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Sci. Total. Environ*, **409**(8): 1444-1452.