Role of Quercetin and L-Arginine in Alleviating Zinc Oxide Nanoparticle Hepatotoxicity in Rats

Nayira A. Abdel Baky* [a], L.M. Faddah [a], Nouf M. Al-Rasheed [a], Nawal M. Al-Rasheed [a] and Wedad Shebali [a]

[a] Pharmacology Department, Faculty of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia.

*Author for correspondence; e-mail: nayiraabelbaky@yahoo.com

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ABSTRACT

The rapid growth of the nanotechnology industry has led to the wide-scale production and application of engineered nanoparticles (NPs). The purpose of this study is to evaluate the toxicity of oral exposure to zinc oxide nano-particles (ZnO-NPs) on liver tissue of Wistar albino rats, and the hepatoprotective effect of quercetin (Qur) and/or L-Arginine (L-Arg) against such ZnO-NPs –induced toxicity. ZnO-NPs were administered orally in two doses (either 600mg or 1g/Kg body weight/day for 5 consecutive days) to rats. In order to detect the protective effects of the studied antioxidants against ZnO-NPs induced hepatotoxicity, biomarkers of metabolic disorder, tissue damage and inflammation, as well as oxidative deoxyribonucleic acid (DNA) damage were investigated. Co-administration of Qur (200mg/Kg body weight) and/or L-Arg (200mg/Kg body weight) daily for three weeks to ZnO-NPs intoxicated rats, with either of the two doses, significantly down-modulated the dramatic alteration in the investigated biochemical parameters. Where, the significant increase in serum alanine amino transferase (ALT) (marker of liver tissue damage), glucose (marker of metabolic disorder) levels, and the level of the pro-inflammatory biomarkers including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), as well as immunoglobin g (IgG) were significantly decreased in treated group as compared to intoxicated rats. Furthermore, the studied antioxidants either alone or in combination effectively ameliorated the hepatic oxidative damage in DNA induced by of ZnO-NPs as confirmed by comet assay. These results support the use of Qur and L-Arg as protective agents against metal oxide nanoparticles hepatotoxicity, with their combination achieving a powerful hepatoprotective action.

Keywords: zinc oxide nano-particles 1, deoxyribonucleic acid 2, tumor necrosis factor-α 3 proinflammatory cytokines, antioxidants

1. INTRODUCTION

Compared with bulk materials, nanoparticles (NPs) have unique and novel properties and thus offer great opportunities for development of new industrial applications [1]. Many NPs are already in use or have potential to be widely used in a range of applications [2]. However, Maynard et al. [3] and Nel et al. [4] have called
for risk assessment for the environment and humans before widespread industrial application of NPs. Such risk assessment requires hazard identification and dose-response data.

Due to their unique properties and diverse nanostructures, ZnO nanoparticles (ZnO-NPs) are widely applied in optoelectronics, cosmetics, catalysts, ceramics, pigments, etc. [5,6]. Previous reports proposed that ZnO-NPs was bio-safe and biocompatible and could be applied in biomedical materials [7]. However, toxicological studies indicated that ZnO-NPs had adverse impacts on human health and environmental species. The bio-safety of ZnO-NPs is still a controversial issue. However, ZnO-NPs were recognized as a respiratory toxicant which caused metal fume fever (myalgia, cough, fatigue, etc.) [8,9]. Moreover, in vivo cell experiments showed that exposure to ZnO-NPs resulted in oxidative damage and inflammation response in vascular/lung endothelial cells [10,11]. Animal experiments also indicated that liver, spleen, heart, pancreas, and bone were also target organs of oral exposure to 20- and 120-nm ZnO [12].

Compared with the conventional toxicology, the dose of ZnO-NPs is no longer a sole factor in evaluating the toxicity of nanoparticles, but the physicochemical properties of its nanoparticles, such as size, shape, chemical composition, aggregation, high specific surface area and its solubility may play a more important role in its toxicity [13-15]. In addition, nanomaterials are theoretically expected to be more toxic than their bulk counterparts due to their greater surface reactivity and the ability to penetrate into and accumulate within cells and organisms [16-18].

The commonly proposed pathogenic mechanisms initiated by NPs are dominated by inflammation-driven effects, including fibrosis, oxidative stress, and DNA damage, making inflammation a target for toxicological testing [1,19,20]. Therefore, new therapeutic antiinflammatory and antioxidant strategies is urgently required. Recently, the effects of several drugs that interfere directly with inflammatory response and oxidative damage have been described [21-23].

Qur (Qur) is the one of the most common phytochemical polyphenolic flavonoids. It has a wide range of reported biological effects including antioxidant, anti-hypertensive, antimicrobial and antiprotozoan activities [22-25]. Qur, is also known as an anti-inflammatory/anti-allergy natural remedy, where it stabilizes mast cell membranes and prevents the release of histamine and other inflammatory agents in the body [26].

On the other hand, L-Arg is one of the most versatile amino acids from the metabolic and physiologic point of view. It has various metabolic and immunologic effects and has been considered to be conditionally essential particularly under inflammatory and oxidative stress condition [27,28]. It can modulate the inflammatory response by modulating the production of inflammatory mediators and cytokine release from activated immune competent cells that play a crucial role in the progression of the pathology [21]. L-Arg can also serve as a substrate for polyamine synthesis, which is known to be strongly involved in protein synthesis enhancement [29], and thus could facilitate healing during inflammation [30].

The aim of this work is to explore the potential protective action of Qur and L-Arg against the metabolic disorder, inflammation and DNA oxidative damage induced by ZnO-NP in rat liver.
2. MATERIALS AND METHODS

2.1. Chemicals

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

2.2. Animals

Ninty male Wistar albino rats weighing 170-200 g were used. The rats were obtained from 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 20-22°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 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 King Saud University, College of Pharmacy.

2.3. Experimental Design

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 used:

Class I, consists of five groups (n=10):

- Group 1 (G1): Normal healthy animals;
- Group 2 to group 5 (G2-G5): Animals administered orally 600mg/Kg/day ZnO-NP for 5 days [15], and divided as follow:
  - G2 : ZnO-NP intoxicated animals with oral low dose (600mg/Kg/day) for 5 days.
  - G3: ZnO-NP intoxicated animals co-administered Qur (Qur) (200mg/Kg/day) [31].
  - G4: ZnO-NP intoxicated animals co-administered L-Arg (L-Arg) (200mg/Kg/day) [32].
  - G5: ZnO-NP intoxicated animals co-administered combination of L-Arg (200mg/Kg/day) and Qur (200mg/Kg/day).

Class II consists of 4 groups; group 6 to group 9 (G6-G9), each of ten rats, administered orally 1g/Kg/day ZnO-NP for 5 days [15], and divided as follow:

- G6 : ZnO-NP intoxicated animals with oral high dose (1g/ Kg /day) for 5days.
- G7: ZnO-NP intoxicated animals co-administered L-Arg (200mg/Kg/day).
- G8: ZnO-NP intoxicated animals co-administered Qur (200mg/Kg/day).
- G9: ZnO-NP intoxicated animals co-administered L-Arg (200mg/Kg/day) and Qur (200mg/Kg/day).

Qur and/or L-Arg were orally administered daily for three weeks from the beginning of the experiment. The body weights of rats were recorded before and after the administration period.

After 24 hours of the last dose administration, rats were sacrificed and the trunk blood was collected. Serum was separated by centrifugation at 3000 r.p.m. for 10 minutes and kept at -80 C for estimations of different biochemical parameters. Liver tissues were collected, rinsed in cold isotonic saline, homogenized, and frozen at -85°C for estimations of DNA oxidative damage using comet assay.
2.4. Serum Biochemical Analysis

2.4.1. Determination of TNF-α level

The concentration of inflammatory cytokines (TNF-α) in serum was determined using commercially available ELISA assays following the instructions supplied by the manufacturer (DuoSet kits, R&D Systems; Minneapolis, MN, USA). The results are shown as pg of cytokine per ml.

2.4.2. Determination of 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 the scattered light is proportional to the size of the aggregates and thus concentration of CRP present in the sample. The intra-assay and interassay coefficients of variation for CRP were 3.3% and 3.2%, respectively. The lower detection limit of the assay was 0.15 mg/L [33].

2.4.3. Determination of IL-6 level

IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with an analytical CV of 6.3% and a detection level of 0.04 pg/mL [34].

2.4.4. Determination of IgG level

IgG level was measured in serum using a sandwich enzyme-linked immunosorbent assay (ELISA). 1 μg/ml of goat anti-rat IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersberg, MD) was used as capture antibodies. Standards were prepared from rat IgG (Sigma Chemical Co., St. Louis, MO) Goat anti-rat.

2.4.5. Determination of ALT

ALT was estimated using kit produced by RANDOX Labs Ltd., UK. The principle for determination of ALT in this kit depend on the reaction of α-oxoglutarate with alanine in the presence of ALT to form L-glutamate plus oxaloacetate. The indicator reaction utilizes the oxaloacetate for kinetic determination of NADH consumption.

2.4.6. Determination of glucose level

Fasting blood glucose was measured according to method adopted previously by Miwa et al. [35] using a glucose kit.

2.5. Comet Analysis in Liver Tissue

Comet assay was used to analyze the level of DNA damage in liver tissues after intoxication with ZnO-NP. The alkaline comet assay was performed according to Singh et al. [36]. The parameters measured to analyse the electrophoretic patterns were: tail length measured from the middle of the head to the end of the tail and relative DNA content in the tail.

2.6. 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 \( p \leq 0.05 \).

3. RESULTS AND DISCUSSION

Several studies have documented high toxicity of ultrafine particles (\( d < 100 \text{ nm} \)) and was associated with respiratory, cardiovascular
and liver diseases [12, 37-40]. It has been demonstrated that NPs, including ZnO-NP, may cause more inflammatory tissue damage than IL-Arger particles of the same materials at a same mass dose delivery [12,39,41,42].

In consistant with previous investigation, the current study revealed that ZnO-NPs induced liver damage as evidenced by the significant elevation of serum ALT (marker of liver tissue damage) and glucose (marker of metabolic disorder) levels in rats intoxicated with low (Figure 1) or high doses (Figure 2) of ZnO-NPs. These findings imply cellular leakage and loss of functional integrity of liver cell membranes, and indicate that liver is one of the tL-Arget organs of such NPs toxicity. Chen et al. [43] and Wang et al. [44] supported our finding as they reported that liver damage could be induced by excess oral zinc salt and zinc powder administration. Moreover, Ding et al. [45], reported that, high dietary zinc caused liver toxicity of mice and resulted in inhibiting the activity of AST in liver homogenate of mice.

**Figure 1.** Effect of Qur and/or L-Arginin treatment on serum ALT (a), glucose (b), and IgG (c) levels in intoxicated rats with low dose of ZnO-NP. Values are expressed as mean ± S.D. ⁴P < 0.001, ³P < 0.01, ²P < 0.05 compared to normal control group, ***P < 0.001 compared to ZnO-NP intoxicated group, **P ≤ 0.01, compared with Qur-treated group, ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 compared with combination group respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.
Coadministration of either Qur and/or L-Arg to ZnO-NPs intoxicated rats with either of the two used dose regimen, significantly reduced the serum ALT level and glucose concentration ($p < 0.001$). The protective effect with the combination of treatment was more effective in reducing the serum enzyme level than Qur alone. This may indicate that the used agents acts as effective hepatoprotective against liver dysfunction caused by nano materials.

Some studies have demonstrated that pathogenic mechanisms initiated by NPs are dominated by inflammation-driven effects, including, oxidative stress, apoptosis and DNA damage [1, 4, 19, 20, 39]. In the present study, it was found that ZnO-NP administration either in low or high dose induced a marked elevation in the the levels of immunological pro-inflammatory biomarkers including TNF-$\alpha$, IL-6, CRP, (Table 1 and 2, respectively) and IgG (Figure 1 and 2, respectively) as compared to normal rats. Meanwhile, the intake of Qur and/or L-Arg along with ZnO-NPs ingestion markedly reduced the elevated IgG ($p < 0.001$), and the sera inflammatory mediators versus intoxicated animals ($p < 0.05$).

Figure 2. Effect of Qur and/or L-Arginin treatment on serum ALT (a), glucose (b), and IgG (c) levels in intoxicated rats rats with high dose of ZnO-NP. Values are expressed as mean $\pm$ S.D. $^aP < 0.001$, $^bP < 0.01$, $^cP < 0.05$ compared to normal control group, $^{***}P < 0.001$ compared to ZnO-NP intoxicated group, $^\&P \leq 0.01$ compared with L-Arg-treated group, compared with Qur treated group, $^{\pi\pi}P \leq 0.01$, $^{\pi}P \leq 0.05$ compared with combination group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.
Table 1. Effect of quercetin and/or arginin treatment on serum inflammatory markers level in intoxicated rats with small dose of n-ZnO particles.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ZnO</th>
<th>Qur</th>
<th>Arg</th>
<th>Qur+Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>242.7±6.1</td>
<td>361.6±9.2a</td>
<td>299.5±3.1b #</td>
<td>300.6±4.1b #</td>
<td>276.12±4.05b</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>3.16±0.1</td>
<td>4.4±0.20a</td>
<td>3.4±0.04∗c</td>
<td>3.1±0.7∗# #</td>
<td>3.3±0.04∗c</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>31±2.098</td>
<td>44.33±1.2a</td>
<td>42.86±0.49 #a</td>
<td>35.66±1.21 #a</td>
<td>35.3±0.81 #a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. of 10 rats, “P ≤ 0.001, “P ≤ 0.01, “P ≤ 0.05 compared with normal group, “P ≤ 0.001 compared with n-ZnO intoxicated group, “P ≤ 0.001, “P ≤ 0.01 compared with combination group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

Table 2. Effect of quercetin and/or arginin treatment on serum inflammatory markers level in intoxicated rats with high dose of n-ZnO particles.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ZnO</th>
<th>Qur</th>
<th>Arg</th>
<th>Qur+Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>242.7±6.1</td>
<td>371.7±6.1a</td>
<td>314.1±2.9b #</td>
<td>322.18±1.9b #</td>
<td>293.6±4.3b</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>3.16±0.1</td>
<td>4.6±0.11a</td>
<td>3.7±0.055∗b #</td>
<td>3.0±0.07#</td>
<td>2.9±0.05#</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>31±2.098</td>
<td>49.57±1.01a</td>
<td>44.94±1.02a #</td>
<td>41.80±0.28a #</td>
<td>39.41±0.62a #</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. of 10 rats, “P ≤ 0.001, ”P ≤ 0.01 compared with normal group, “P ≤ 0.001 compared with n-ZnO intoxicated group, “P ≤ 0.05, “P ≤ 0.01, “P ≤ 0.01 compared with combination group, respectively, using ANOVA followed by Bonferroni as a post-ANOVA test.

TNF-α is one of the most common inflammatory injurious chemokine immunological markers increased in response to different metal oxide NPS-toxicity including ZnO [46,47]. The up-regulation of this cytokine triggers the production of other cytokines as IL-6, that end with inflammatory liver injury [48]. As IL-6 triggers the activation of transcription factors that bind to DNA elements and stimulate increased transcription of CRP, resulting in a rise in its serum level [48,49]. CRP is a member of the pentaxin protein family involved in pattern recognition and innate immunity; it is synthesized primarily by the liver in response to inflammation [50]. 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 [51]. It also has a principle role in the activation of proinflammatory pathways in various cell types [52,53]. All of the previous data are in line with our results in which inflammatory markers and serum glucose level were significantly increased in intoxicated groups.

The marked increase in the circulating IgG in rat sera intoxicated with either doses of ZnO-NPs is another response induced by this NPs toxicity. It was suggested that the increase in the circulating antibody production is the result of different inflammatory cytokines production including TNF-α with potential impact on immunoglobulin
production during inflammation [54]. These result may indicate that ZnO-NPs induced inflammatory liver injury through production of the inflammatory mediators.

Thus, a protective strategy attenuates the production of inflammatory mediators could ameliorate both liver injury and remote organ dysfunction. The intake of Qur or L-Arg or their combination immediately with ZnO-NPs ingestion presented in this study was beneficial in the prevention of this NPs induced inflammatory liver injury. The protective actions of these agents against ZnO-NPs hepatotoxicity in rats may be attributed to their abilities in inhibiting inflammatory mediators expression. Previous studies showed that Qur reduced inflammatory pain by inhibiting oxidative stress and cytokine production in Swiss mice, suggesting that Qur possesses anti-inflammatory effects in vivo [23, 55]. Also, previous publication reported that, the metabolites of Qur possess antiinflammatory properties. Additionally, quercetin-32-sulfate, one of the major metabolites inhibits the production of pro-inflammatory cytokines in A549 cells induced by IL-1 [56]. It has been also shown that, Qur reduce IL-6 and TNFα release in LPS-challenged murine macrophages [26]. Moreover, Tribolo et al. [57] showed that quercetin-sulfate, quercetin glucuronides, and methylquercetin-3-glucuronide inhibit the expression of key molecules, such as vascular cell adhesion molecule-1, that regulate monocyte recruitment in atherosclerosis. Furthermore, a decrease in oxidative stress by Qur metabolites may also be involved in such anti-inflammatory effects of Qur supplementation. Several studies have shown that Qur metabolites may act as antioxidants [58]. On the other hand, it was found that L-Arg supplementation protects against oxidative damage and inflammatory responses caused by different pathological conditions [27, 59]. Growing evidence indicates the beneficial role of nutrient mixture L-Arg and Qur in inhibiting the inflammatory response in experimental inflammation by down-regulating the pro-inflammatory cytokine protein expression [28].

Penetration of NPs into the nucleus has been shown in a number of studies [(60,616),(645,825)]. Some authors have shown that 1.4 nm Au55 particle clusters interact with DNA in a way which may be the reason for the strong toxicity of these tiny particles towards human cancer, since it is generally known that DNA double-strand breaks may cause cancer [61]. The comet assay is considered to be a rapid, sensitive and a relative simple assay for detecting DNA damage at the level of individual cells [36]. Increased DNA migration results from the induction of DNA single-strand breaks, alkali-labile sites, and incomplete excision repair sites at the time of Lysis. Increased DNA migration also accompanies the DNA fragmentation associated with the cell death arising from a non-DNA-mediated process or apoptosis [62]. 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, reflect DNA damage, though the percentage of tail DNA generally seems to be the most useful, as it is directly related to the frequency of breaks over a wide range of damage [63].

The use of the very sensitive single-cell electrophoresis assay to detect DNA damage indicated that ZnO-NPs induced liver DNA damage as documented by a significant increase in the tail length and DNA % in the tail from liver samples of rats intoxicated with either of the two doses of ZnO-NPs.
(Figures 3, 4 and 5). This effect was sever in rat livers ingested the high dose of the ZnO-NPs. The DNA damaging potential of ZnO-NPs was previously documented in human epidermal cells [64]. Also, the present data is supported by a study conducted by Dufour et al. [65] who have shown a concentration related increase in chromosome aberrations upon ZnO-NPs exposure (<100 nm) in CHO cells although at a very high concentration (≥ 105 μg/ml).

**Figure 3.** Tail length and DNA percentage in tail of comets obtained from liver tissue of intoxicated rats with low (a,b) and high dose (c,d) of ZnO-NP particles, and the effect of Qur and/or L-Arginin treatment on reduction of DNA damage. Values are means ±S.D (n=10). aP<0.001, bP < 0.01, cP < 0.05 compared to normal control group, ***P < 0.001, **P < 0.01, *P < 0.05 compared to ZnO-NP intoxicated group, $P ≤ 0.05$ compared with Qur-treated group, using ANOVA followed by Bonferroni as post ANOVA test.

**Figure 4.** DNA damage in the liver tissue in intoxicated rats with low dose of ZnO-NP, and the effect of Qur and/or L-Arginin treatment on the level of DNA damage. Comet assay showing degree of DNA damage in liver tissue of (a) normal control group, (b) group intoxicated with low dose of ZnO-NP particles, (c) intoxicated group treated with Qur, (d) intoxicated group treated with L-Arg, and (e) intoxicated group treated with Qur and L-Arg.
As there is a well documented link between NPs and oxidative stress, one of the possible modes that can be suggested for ZnO-NPs induced DNA damage may be lipid peroxidation and oxidative stress [39]. Reactive oxygen species (ROS) are known to react with DNA molecule causing damage to both purine and pyrimidine bases as well as DNA backbone [66]. Another important outcome of ROS production is lipid peroxidation which generates a variety of products reactive towards cellular macromolecules including DNA. Malondialdehyde, one of the major products of lipid peroxidation, is a proven mutagen and carcinogenic compound which reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine and deoxycytidine [67,68]. DNA damage resulting from any of these probable mechanisms may trigger signal transduction pathways leading to apoptosis or cause interferences with normal cellular processes thereby causing cell death [64].

Coadministration of Qur and/or L-Argin in treated ZnO-NPs intoxicated rats with either of the studied two doses effectively protected their livers from DNA damage. In vitro and animal studies have shown that Qur possess anti-inflammatory, antioxidant, hepato-protective, and anticarcinogenic activities [69]. The flavonoids inhibit the enzymes responsible for $O_2^\cdot$ production [70], the low redox potentials of flavonoids thermodynamically allow them to reduce highly oxidising free radicals such as $O_2^\cdot$, RO$^\cdot$ and HO$^\cdot$ [71], and a number of flavonoids chelate trace metals [72]. Besides scavenging, flavonoids may stabilise free radicals by complexing with them [73]. Qur, the most abundant flavonoid in the human diet [74] was investigated against the formation of oxidative DNA damage both in vitro and in vivo in the Comet assay. A significant dose-

Figure 5. DNA damage in the liver tissue in intoxicated rats with high dose of ZnO-NP, and the effect of Qur and/or L-Argin treatment on the level of DNA damage. Comet assay showing degree of DNA damage in liver tissue of (a) normal control group, (b) group intoxicated with high dose of ZnO-NP, (c) intoxicated group treated with Qur, (d) intoxicated group treated with L-Arg, and (e) intoxicated group treated with Qur and L-Arg.
dependent protection by Qur against the formation of oxidative DNA damage generated by H$_2$O$_2$ was observed in vitro [75]. Also, L-Arg has been shown to ameliorate oxidative stress in the liver and brain tissues and has antioxidant activity [76], as well as ROS-scavenging properties [77]. External supplementation with L-Arg was able to inhibit DNA fragmentation [78]. Moreover, it has been shown that L-Arg supplemented diet increased wound DNA synthesis [79]. The increased synthesis of wound DNA may be attributable to the unique function of L-Arg in cell proliferation. This function is linked to polyamines (which are essential for cell proliferation and differentiation) production via the conversion of L-Arg to ornithine by L-Arginase [29, 80].

**CONCLUSION**

In the current study, the hepatotoxic potential of ZnO-NP was evidenced from the elevation in inflammatory cytokines (TNF, IL6, and CRP), as well as the significant increase in serum ALT and glucose levels. Additionally, ZnO-NP induced oxidative DNA damage in liver cells, which further indicate the hepatotoxic potential of ZnO-NP. Treatment with either L-Arg, Qur, or their combination successively alleviated the alterations in the previous biomarkers, as well as effectively reduced liver cells DNA damage in ZnO intoxicated rats. This may be related to their ability to attenuate the extent of liberation of ROS and inflammatory cytokines induced by such nanoparticles. All of these findings suggest that, supplementation of Qur and L-Arg as prophylactic agents may be beneficial against liver injury and oxidative DNA damage caused by metal oxide nano-particles. In addition, their collective combination may be helpful in combating various hepatic diseases.

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