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Ameliorative Effects of Curcumin Nanoparticles on Hepatotoxicity Induced by Zearalenone Mycotoxin

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Abstract: Curcumin has potent anticancer effects in different cancer types. However, the therapeutic applications of curcumin are limited due to its high metabolic instability, poor absorption and bioavailability. Zearalenone (ZEN) is a mycotoxin has hepatotoxic effects and induces adverse liver lesions. The aims of the current study were to prepare curcumin nanoparticles (CurNPs) and to evaluate its protective role against ZER-induced hepatotoxicity in rats. Male Sprague-Dawley rats were divided into 6 treatment groups and treated for 3 weeks as follow: the control group, the groups treated with low dose (100 mg/kg b.w) or high dose (200 mg/kg b.w) of CurNPs and the groups treated with ZEN (40 µg/kg b.w.) alone or in combination with CurNPs at the two tested doses. Blood and tissue samples were collected for different biochemical, cytological analyses and histological examination. The prepared CurNPs showed an average size of 100.5 ± 40 nm and negative zeta potential value of 27.5 ± 3.43 mV at natural pH. The results of the biological study revealed that treatment with ZEN alone resulted in a significant increase in liver function parameters, lipid peroxidation (MDA) and DNA fragmentation accompanied with a significant decrease in body weight gain, antioxidant capacity and down regulation of GPX mRNA gene expression as well as severe histological changes in the hepatic tissue. Animals treated with CurNPs at the two tested doses were comparable to the control group. The combined treatment with CurNPs at the two tested doses plus ZEN resulted in a significant improvement in all tested parameters and histological picture of liver tissue in a dose-dependent manner. It could be concluded that CuNPs could overcome the problems associated to the poor solubility of curcumin and succeeded to induce a potential protection against hepatotoxicity induced by ZEN mycotoxins.

Key words: Zeralenone · Curcumin · Curcumin nanoparticles · Hepatotoxicity · Oxidative stress

INTRODUCTION

Zearalenone (ZEN) is a secondary metabolite produced mainly by the fungal species *Fusarium graminearum* and *Fusarium culmorum* and others [1-3]. ZEN is one of the most toxic mycotoxins found worldwide not only in maize, oats, rice, rye, sorghum and wheat [4, 5]. It is non-steroidal, estrogenic mycotoxin that has a role as a mammalian endocrine disrupter in different species [6]. Moreover, ZEN is hepatotoxic and induces adverse liver lesions [7, 8]. Therefore, ZEN contaminated products pose a risk to animal and human health if they are used as foodstuff. In the long term, ZEN can cause nontrivial economic damage to the livestock industry and human health [9]. ZEN is mainly metabolized in the liver which seems to be a main target [10]. Thus, ZEN was found to be hepatotoxic and to induce liver lesions [7, 11]. Several studies have been conducted and have shown that ZEN is cytotoxic, inhibits cell proliferation and macromolecules synthesis in different cell lines [12, 13] and exhibits a geneotoxic potential through induction of micronuclei, chromosome aberrations, DNA fragmentation, cell cycle arrest, etc. [14-18].

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Curcumin is a natural phenolic compound isolated from the herb *Curcuma longa* (turmeric) [19]. Several reports were discussed the health beneficial properties of curcumin such as antioxidant, anti-inflammatory, anti-cancer and many others [20-22]. However, the activities of curcumin are not fully realized due to its low water solubility, low stability and low bioavailability [23]. To solve these problems of curcumin, researchers have explored several ways such as the nano-system and pro-drug strategies which attracted much attention [24]. The aims of the current study were to prepare curcumin nanoparticles (CurNP) and to evaluate its protective role against ZER-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals and Kits: Zearalenon was purchased from Sigma-Aldrich, USA. And curcumin was purchased from Mepaco Arabian Pharmaceutical, Cairo, Egypt. Transaminases (ALT and AST), alkaline phosphatase (ALP) and albumin kits were purchased from Randex Laboratories (San Francisco, CA, USA).Glutathione peroxidase (GPx), lipid peroxidation (MDA) and Alfa feto protein (AFP) kits were purchased from Biodiagnostic Co. (Giza, Egypt). Catalase (CAT) and total antioxidant capacity (TAC) kits were obtained from Eagle diagnostics (Dallas, TX, USA). TRIzol reagent was purchased from Molecular Research Center, Inc. (Cincinnati, OH, USA) and RNA Fermentas kit was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

Preparation of Curcumin Nanoparticles (CurNPs): CurNPs were prepared according to the method described by Li *et al*. [24]. In brief, 100 mg of curcumin (0.27 mmol) was dissolved in 20 ml dichloromethane and 1 ml of this solution was sprayed into 50 ml boiling water drop wise with a flow rate of 0.2 ml/min for 5 min under ultrasonic conditions using an ultrasonic power of 100 W and a frequency of 30 kHz. The contents were stirred at 200-800 rpm at room temperature for 20 min after sonication for 10 min when a clear orange-colored solution was obtained. The solution was concentrated under reduced pressure at 50°C and then freeze-dried to obtain an orange powder. Particle sizes were determined using dynamic light scattering (DLS; Zetasizer™ 3000E, Malvern Instruments Worcestershire, UK). Zeta potential measurement was based on nanoparticle electrophoretic mobility and calculated form *Smoluchowski's* equation [25]. All measurements were performed in triplicate at 25°C. Morphological determination of CurNPs was analyzed by transmission electron microscopy (2100-HR, JEOL, CA, USA). Briefly, a drop of the fresh CurNPs sample was placed onto a carbon-coated copper grid, forming a thin liquid film, which was negatively stained by addition of a drop of uranyl acetate. The excess of the staining solution was removed with filter paper then air-dried before the observation. Image acquisition was done with an Orius 1000 CCD camera (GATAN, Warrendale, PA, USA).

Experimental Animals: Three-months old Sprague-Dawley male rats (140-160 g) were purchased from Animal House Colony, Biochemical laboratory, Faculty of Medicine, Cairo University, Egypt and were maintained on standard lab diet (protein: 160.4; fat: 36.3 and fiber 41g/kg). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., Faculty of Medicine, Cairo University, Egypt. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the Faculty of Medicine, Cairo University, Giza, Egypt.

Experimental Design: Animals were divided into six groups (8 rats/group), housed in filter-top polycarbonate cages and were maintained on their respective diet for three weeks as follow: group 1, normal control animals; group 2, rats treated orally with low dose of CurNPs (100 mg/kg b.w) in distilled water (CurNPs-LD); group 3, rats treated orally with high dose of CurNPs (200 mg/kg b.w) in distilled water (CurNPs-HD); group 4, rats treated orally with ZEN (40 µg/kg b.w.) in corn oil; group 5, rats treated with ZEN plus CurNPs-LD and group 6, rats treated with ZEN plus CurNPs-HD. The animals were observed daily for any signs of toxicity and body weight was recorded twice a week. At the end of the treatment period (i.e. day 21) all animals were fasted for 12 h, then blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia. Sera were separated using cooling centrifugation and stored at -20°C until analysis. The sera were used for the determination of ALT, AST, ALP, albumin, total protein and AFP according to the kits instructions.

After the collections of blood samples, animals were sacrificed and samples of the liver of each animal were dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20%, w/v homogenate according to Lin *et al*. [26]. This homogenate was centrifuged at 1700 rpm at 4°C for 10 min; the supernatant was stored at -70°C until analysis. This supernatant (20%) was used for the determination of hepatic lipid peroxidation (MDA) and it

was further diluted with phosphate buffer solution to give 2 and 0.5% dilutions for the determination of hepatic GPx (2%) and CAT (0.5%) and TAC according to the kits instructions. Other samples of liver each animal were dissected for molecular analyses and histological examination.

DNA Fragmentation Assays for Apoptosis: Apoptotic changes in the liver were evaluated colorimetrically by DNA fragmentation and by agarose gelelectrophoresis according to the procedure of Perandones *et al.* [27]. Liver samples were homogenized in 700 µl hypotonic lysis buffer (10 mM Tris base, 1 mM EDTA and 0.2 %Triton X-100) and centrifuged for 15 min at 11, 000 rpm. The supernatants containing small DNA fragments were separated; one-half of the volume was used for gel electrophoresis and the other half together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the diphenyl amine (DPA) assay. The samples were treated with equal volumes of absolute isopropylalcohol and NaCl to precipitate DNA. Extracted DNA was electrophoresed on 1% agarose gels containing 0.71 µg/ ml ethidium bromide. At the end of the runs, gels were examined using UV transillumination. The diphenyl amine (DPA) assay reaction suggested by Burton [28] and modified by Perandones *et al*. [27] was applied. The colorimetric reaction was measured spectrophotometrically at 575 nm and the percentage of DNA fragmentation was calculated.

Determination of GPX Gene Expression in Liver

RNA Extraction: Liver tissue cells were ground in liquid nitrogen and total RNA was extracted from all experimental animals. The extraction of total RNA was performed using TRIzol reagent according to the manufacturer's procedures. The concentration and purity of RNA was measured at 260/280 nm using Ultraviolet spectrophotometer (ratios fell between 1.75 and 1.9, indicating very pure RNA in all cases). Equal amounts of RNA isolated from individual rat of each group were prepared for the semi-quantitative RT-PCR [29].

Semi-Quantitative Reverse Transcription and PCR Reaction: The first-strand cDNA was prepared from the 5 µg of total RNA using Fermentas kits. The used RT program was: 60 min at 42°C (cDNA synthesis); 5 min at 94°C (denaturation). Afterwards the reaction tubes containing RT preparations were ash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR) [30]. The first-strand cDNA from different rat samples was used as the template for amplification by the PCR with the following pairs of specific primers (from 5' to 3'): GPX forward: CTCTCCGCGGTGGCACAGT, GPx reverse: CCACCACCGGGTCGGACATAC [31]. β-actin forward: CGTGACATTAAGGAGAAGCTGTGC, β-actin reverse: CTCAGGAGGAGCAATGATCTTGAT a house-keeping gene, was used for normalizing mRNA levels of the target genes [32]. The PCR cycling parameters were one cycle of 94°C for 5 min, 35 cycles of 94°C for 30 s, 60°C (GPX genes) for 30 s, 70°C for 40 s and a final cycle at 72°C for 5 min. The PCR products were electrophoresed onto an ethidium bromide stained 2.0% agarose gel. The ethidium bromide-stained gel bands were scanned and the signal intensities were quantified by the computerized Gel-Pro program image analyzer (Version 3.1 for Windows3).

Statistical Analysis: All data were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System [33]. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio [34]. All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

Transmission electron microscopy (TEM) image (Fig. 1) showed nearly spherical shape for the prepared CurNPs with total volume ranged from 102 x 119 and 109 x 129 nm. The size distribution of CurNPs by DLS measurements expressed in numbers showed an average size of the particles is 100.5 ± 40 nm (Fig. 2). The CurNPs also showed a negative zeta potential value of 27.5 ± 3.43 mV at pH 7.

The effect of ZEN and CurNPs at the two tested doses alone or in combination on daily body weight (Fig. 3) showed that insignificant difference in body weight gain between the control group or those treated with CurNPs-LD or CurNPs-HD alone or in combination with ZEN. However animals treated with ZEN alone showed a significant decrease in body weight gain started from day 5 of treatment till the end of experiment.

The current results revealed that rats treated with ZEN showed a significant increase in ALT, AST and ALP accompanied with a significant decrease in serum albumin (Table 1). Animals treated with CurNPs at the two tested doses were comparable to the control group. However, the combined treatment with CurNPs-LD succeeded to normalize ALP and albumin and CurNPs-HD succeeded to normalize all these parameters (Table 1).

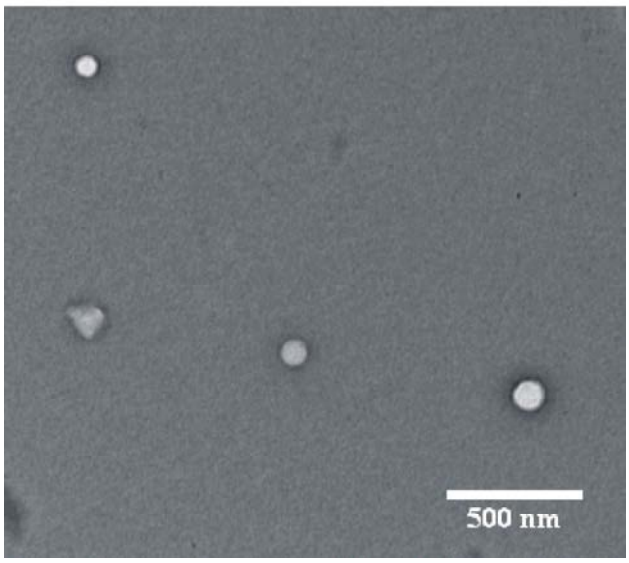

Fig. 1: TEM image of the prepared CurNPs

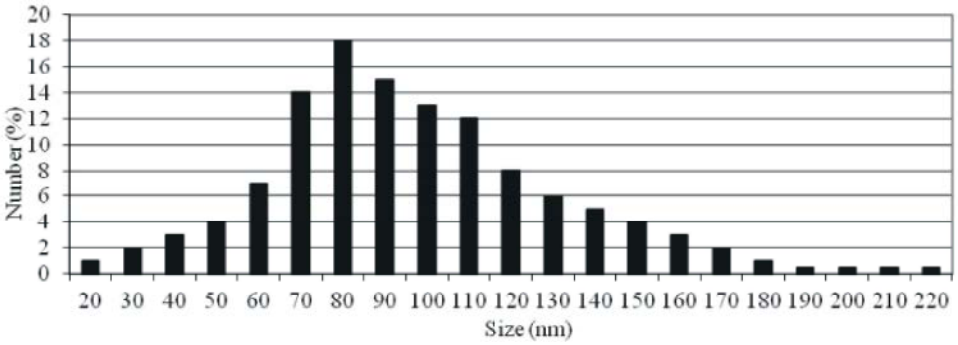

Fig. 2: The size distribution of CurNPs by DLS measurements

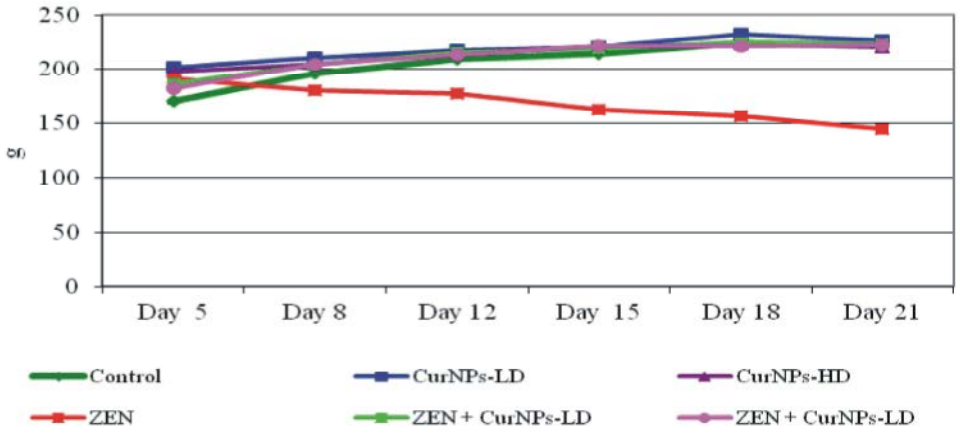

Fig. 3: Effects of CurNPs on changes of daily body weight gain in rats treated with ZEN

The data presented in Table (2) revealed that CurNPs at the two tested doses induced insignificant increase in GSH, CAT and TAC accompanied with insignificant decrease in MDA. Animals treated with ZEN alone showed a significant decrease in the antioxidant enzymes activities and a significant increase in MDA. The combined treatment with CurNPs at the two tested doses plus ZEN resulted in a significant improvement in the oxidative stress markers. This improvement was more pronounced in the group received CurNPs-HD plus ZEN.

Table 1: Effects of CurNPs on serum ALT, AST, ALP and Albumin activities in rats treated with ZEN

Parameters Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)
Control	28.1 ± 0.82[a]	26.54 ± 0.62[a]	34.12 ± 1.46[a]	4.82 ± 0.35[a]
CurNPs-LD	28.87 ± 0.51[a]	26.8 ± 0.49[a]	35 2.11[a]	5.67 ± 0.23[b]
CurNPs-HD	28.91 ± 0.45[a]	26.037 ± 0.66[a]	31.38 ± 2.83[a]	4.94 ± 0.25[a]
ZRN	44.15 ± 3.55[b]	58.08 ± 2.05[b]	47.63 ± 2.27[b]	2.69 ± 0.26[c]
ZRN + CurNPs-LD	31.75 ± 3.21[c]	33.59 ± 0.69[c]	33.63 ± 1.52[a]	4.27 ± 0.18[a]
ZRN + CurNPs-HD	29.21 ± 1.29[a]	28.01 ± 0.40[a]	34.5 ± 1.43[a]	4.14 ± 0.31[a]

Means superscript with different letter are significantly different ($P \leq 0.05$)

CurNPs-LD: Low dose of curcumine nanoparticles (100 mg/ kg. b.w); CurNPs-HD: High dose of curcumine nanoparticles (200 mg/kg b.w); ZRN: Zearalenone (40 µg/ kg. b.w)

Table 2: Effects of CurNPs on hepatic oxidative stress markers in rats treated with ZEN

Parameters Groups	GSH (mg/tissue)	CAT (nmol/g tissue)	TAC (mM/g tissue)	MDA (nmol/g tissue)
Control	52.84 ± 1.44[a]	2.56 ± 0.18[a]	4.97 ± 0.41[a]	1.31 ± 0.15[a]
CurNPs-LD	55.74 ± 1.71[a]	2.44 ± 0.15[a]	4.51 ± 0.47[a]	1.03 ± 0.05[a]
CurNPs-HD	57.58 ± 1.36[a]	2.73 ± 0.22[a]	5.55 ± 0.34[b]	1.25 ± 0.03[a]
ZRN	21.26 ± 1.15[c]	0.31 ± 0.04[c]	1.91 ± 0.37[c]	11.63 ± 0.69[b]
ZRN + CurNPs-LD	41.3 ± 1.96[d]	1.06 ± 0.07[d]	4.04 ± 0.38[a]	5.72 ± 0.65[c]
ZRN + CurNPs-HD	48.99 ± 2.05[b]	1.67 ± 0.16[e]	4.96 ± 0.41[e]	3.09 ± 0.38[d]

Means superscript with different letter are significantly different ($P \leq 0.05$)

CurNPs-LD: Low dose of curcumine nanoparticles (100 mg/ kg. b.w); CurNPs-HD: High dose of curcumine nanoparticles (200 mg/kg b.w); ZRN: Zearalenone (40 µg/ kg. b.w)

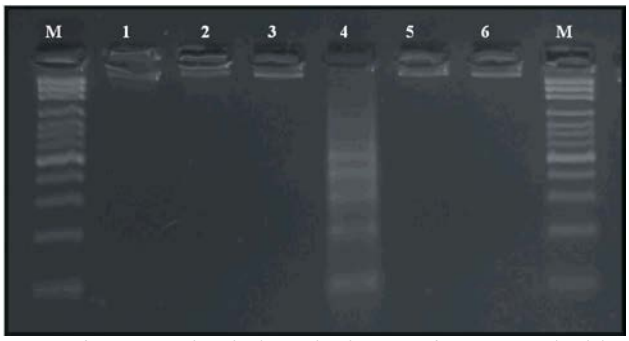

Fig. 4: Effects of CurNPs on DNA fragmentation in hepatic tissues of rats treated with ZEN. Lane M: the DNA marker (100 pb), Lane 1: Control, Lane 2: CurNPs-LD, Lane 3: CurNPs-HD, Lane 4: ZEN, Lane 5: ZEN + CurNPs-LD and Lane 6: ZEN + CurNPs-LD

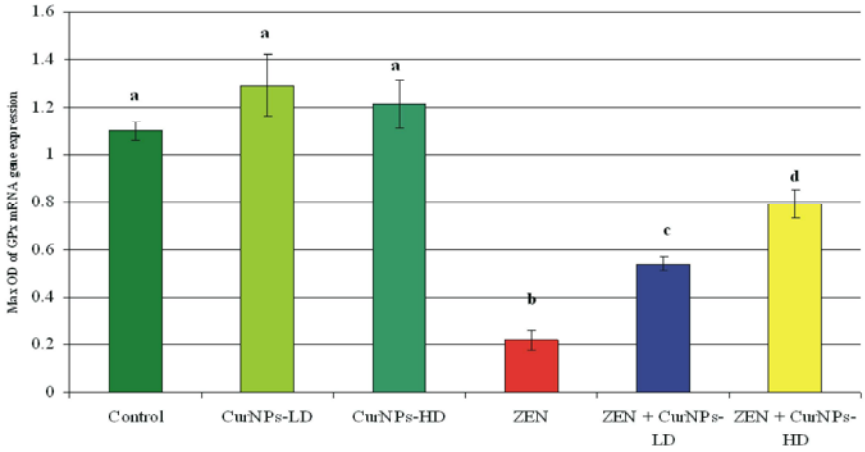

Fig. 5: Effects of CurNPs on GPX mRNA expression in liver of rats treated ZEN

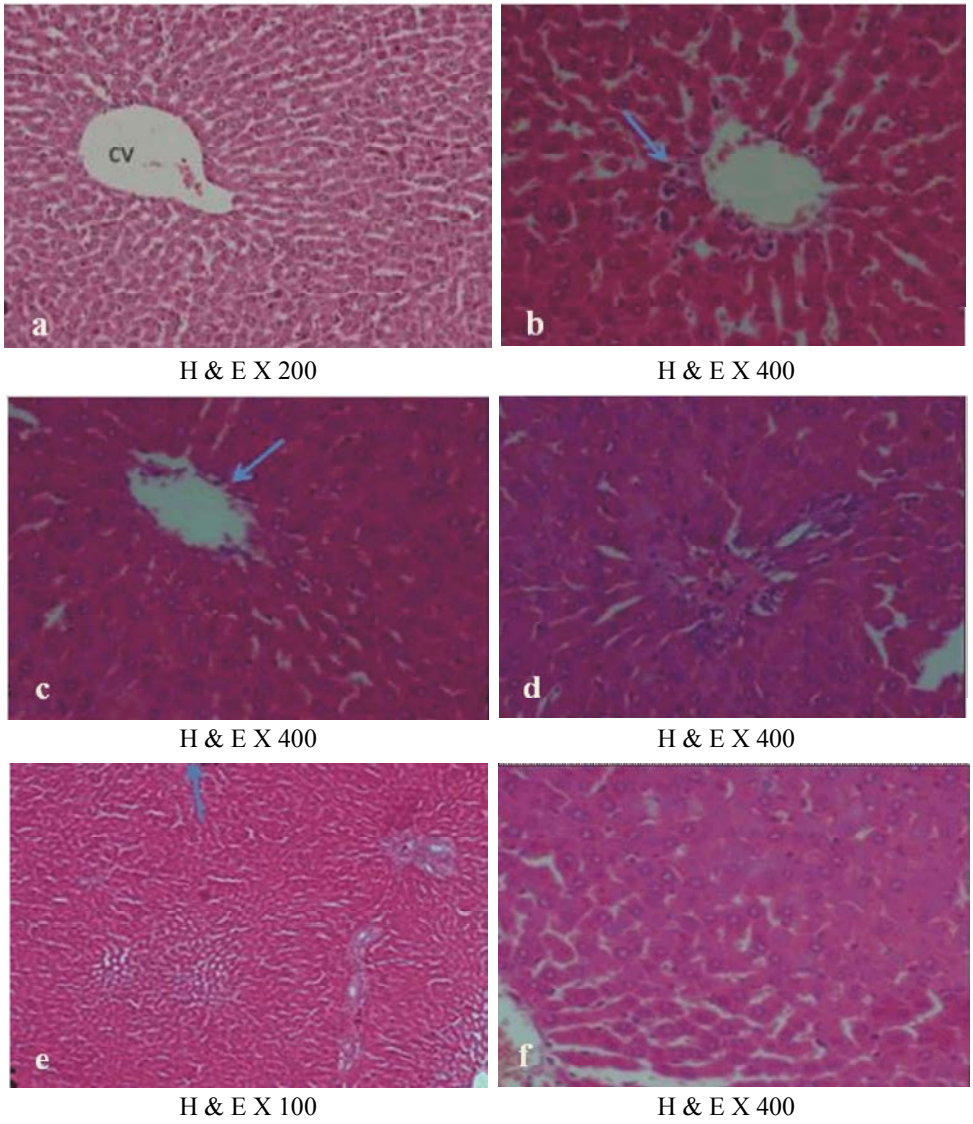

Fig. 6: Photomicrograph of liver section of (a) control rats showing the normal hepatocytes with vesiculated nuclei and central vein; (b) rats treated with CurNPs-LD or (c) rats treated with CurNPs-HD showing nearly normal hepatocytes with vesiculated nuclei. some mononuclear cellular infiltration around the central vein also seen; (d) rats treated with ZEN showing marked necrosis and fibrous tissues around thick walled portal tracts; (e) rats treated with ZEN plus CurNPs-LD showing nearly normal hepatocytes with few fibrosis around the portal tract and (f) rats treated with ZEN plus CurNPs-HD showing nearly normal hepatocytes with focal necrosis and fibrosis around the blood vesseles and interstitial haemorrhage

Treatment with ZRN alone resulted in marked increase in DNA fragmentation since necrotic strand breaks/streaking DNA was observed in this group (Fig. 4, lane 4). Rats treated with CurNPs at the two tested doses did not show any significant DNA fragmentation. However, the combined treatment with ZEN plus CurNPs at the low or high doses succeeded to decrease DNA fragmentation induced by ZEN. Moreover, GPX mRNA gene expression was significantly decreased in the group treated with ZEN alone (Fig. 5). However, up-regulation of GPX mRNA was observed in the groups received CurNPs-LD and CurNPs-HD. Moreover, these treatments succeeded to improve GPX mRNA gene expression which was more pronounced in the group treated with the high dose of CurNPs plus ZEN (Fig. 5).

The microscopic examination of the liver tissues of the control rats showed normal hepatocytes with vesiculated nuclei and central vein (Fig. 6a). Rats treated with CurNPs-LD or CurNPs-HD showed nearly normal hepatocytes with vesiculated nuclei with some mononuclear cellular infiltration around the central vein (Figs. 6b, c). The examination of liver tissue in animals treated with ZEN alone showed marked necrosis and fibrous tissues around thick walled portal tracts (Fig. 6d). Rats treated with ZEN plus CurNPs-LD showed nearly normal hepatocytes with few fibrosis around the portal tract (Fig. 6e) and the liver of rats treated with ZEN plus CurNPs-HD showed nearly normal hepatocytes with focal necrosis and fibrosis around the blood vessels and interstitial haemorrhage (Fig. 6f).

DISCUSSION

Curcumin has extremely low water solubility that limiting its potential applications in food and pharmaceutical industries. According to Kurien *et al.* [35], the solubility of curcumin in water is about 0.6 μg/ml. The preparation of CurNPs may enhance the water solubility of curcumin since the decrease in particle size resulted in the formation of a thinner hydrodynamic layer around particles which increase the surface specific dissolution rate [36].

In the current study, the DLS analysis of the aqueous dispersion of the prepared CurNPs showed the formation of nanoparticles with an average hydrodynamic diameter of 105 ± 40 nm with spherical shape as shown by the electron micrographs. The results of particle size and shape reported in the current study were in agreement with the previous work of other investigators using different preparation techniques [37-39]. In this concern anderberg *et al.* [40] suggested a hyperbolic relation between the particle size and the surface specific dissolution rate that increase the solubility due to their larger surface area which promotes dissolution [41]. Similar to the current results, Kesisoglou *et al.* [42] reported that reduction in the particle size of active ingredients to nanoparticles size has shown improvement in solubility and bioavailability. On the other hand, the prepared CurNPs showed a negative zeta potential (27.5 ± 3.43 mV) at natural pH. This high negative charge also ensures the colloidal stability of CurNPs as suggested by Li *et al*. [24].

In the present study, the protective role of CurNPs was evaluated against the hepatotoxiciy of ZEN mycotoxins. The selected dose of ZEN was based on our previous work [15] however; the selected dose of CurNPs was based on the work of Sankar *et al.* [43]. The results revealed that ZEN showed hapatotoxicity in rats as indcated by the significant decrease in body weight gain and increasing of hepatic marker enzymes (ALT, AST and ALP), albumin and MDA accompanied with the significant decrease in hepatic antioxidants (GSH, CAT and TAC) and GPX mRNA gene expression as well as the histological changes in the liver tissues.

Previous studies reported that ZEN is resorcylic acid lactones and functionally is mycoestrogen has the ability to increase the incidence of adenomas in pituitary and liver [44]. Treatment with ZEN induced potential hepatotoxicant which may be due to its estrogenic property. The impaired hepatic function in ZEN-treated rats suggested the activation of hepatocytes led to increase hepatic marker enzymes. On the other hand, hepatic injury is complex and the hepatotoxin activates the hepatic cells resulting in free radical- mediated tissue injury by series of chain reactions resulting in lipid peroxidation.

ZEN is metabolized by the cellular cytochrome P450 enzyme system to a reactive intermediate, which reacts with macromolecules such as lipids, nucleic acid and protein, leading to lipid peroxidation and cellular injury [12, 44]. On the other hand, Frizzell *et al*. [45] reported that ZEN and its metabolites have potential endocrine disruptors by altering hormone production and ZEN ingestion decreased TNF-α and IL-8 synthesis [46]. Moreover, ZEN derivatives (α-ZOL, β-ZOL, ZAN) showed similar immunosuppressive effects in swine [47]. Taken together, ZEN showed multiple side effects indicated its hepatotoxic effects.

ZEN-induced alterations in the hepatic antioxidant status may therefore be considered as manifestation of increased oxidative stress caused by ZEN and/or its metabolites. Both GSH and CAT are considered to be enzymatic free-radical scavengers in the cells. In the present study, ZEN alone decreased GSH and CAT in the liver suggesting that these enzymes may be conjugated with ZEN or its metabolites since the detoxification of ZEN can be mediated by GSH *S*-transferase-catalyzed conjugation in the liver [48]. On the other hand, the decrease in TAC and the increase in MDA level in the liver of the ZEN-treated rats might indirectly lead to an increase in oxidative DNA damage [49, 50]. Moreover, the reduction of TAC level may be explained by the conjugation of glutathione peroxidase (GPX) with ZEN or its metabolites [51]. On the other hand, Abid-Essafi *et al*. [12] and Azeredo-Martins *et al*. [52] stated that the decrease in SOD in rats treated with ZEN lead to an indirect increase in oxidative DNA damage.

The increase in DNA fragmentation reported in the current study revealed the genotoxicity of ZEN and confirmed the results of Abbès *et al*. [12] and Abid-Essafi *et al.* [15] who reported that ZEN induce apoptosis through three linked processes including sustained DNA injury, DNA lesions or DNA fragmentations and cell cycle arrest in the G2/M phase. Furthermore, it was suggested that ZEN damaged DNA repair capability and initiated the apoptosis pathway [53]. On the other hand, the decreased in GPX mRNA gene expression reported herein confirmed the genotoxicity of ZEN. The histological examination of the liver section in rats treated with ZEN showed marked necrosis and fibrous tissues around thick walled portal tracts. These results confirmed the biochemical results and suggested the hepatotoxicity of ZEN. Similar observations were reported in previous studies [8, 48].

The protective role of CurNPs was manifested by the significant improvement in all biochemical, the cytogenetical parameters tested and the histological results. CurNPs alone at the two tested doses did not induce any toxicity. In contrary, it showed an antioxidant effect as since it increased the antioxidant enzymes activity, TAC and reduced MDA especially at the high dose. The antioxidant activity and the ability to scavenge free radicals, inhibits lipid peroxidation and prevents DNA damage as well as improves endogenous antioxidants defenses of curcumin is well documented [54, 55]. It is also has the ability to neutralize chemical carcinogens such as superoxide, peroxyl, hydroxyl radical and nitric oxide radical constitutes [56]. Previous reports suggested that curcumin is a multifunctional molecule has three functional groups that contribute to its biologic activity. These groups include: an aromatic *o*-methoxy phenolic group, α, β-unsaturated β-diketo moiety and a seven carbon linker [56]. Curcumin is suggested to act through a number of targets and metabolic pathways involving transcription, cell growth and apoptosis [57, 58]. Moreover, these authors reported that it practically non-cytotoxic to normal human cells but cytotoxic for cancer cells and inhibits oncogenic cell proliferation by arresting cell cycle progression and induction of apoptosis. However, the main limiting factors are their low aqueous solubility, poor bioavailability, high rate of metabolism and decomposition [59, 60] which were overcome in the current study through the application of nanotechnology.

In the current study, the prepared CurNPs showed a high antioxidant activity at a dose as low as 100 mg/ kg b.w. The pharmacokinetic, biodistribution and therapeutic efficacy of different CurNPs have been investigated in many animal studies to evaluate the potential role for the treatment of different diseases. Khalil *et al*. [61] showed that CurNPs displayed better pharmacokinetics profiles compared to a curcumin aqueous dispersion after a single oral dose of 50 mg/kg in rats. The mean half-lives of CurNPs were 4 and 6 h, respectively, compared to a half-life of free curcumin of 1 h [62]. According to Khalil *et al*. [61], the better performance was due to the lack of interaction of CurNPs with digestive enzymes present in the gastrointestinal tract as a possible reason for the better bioavailability. Moreover, CurNPs might be taken up by M cells of Peyer's patches or might be taken by enterocytes via a transcellular mechanism [63, 64]. Several studies have demonstrated that curcumin, particularly in the nano form, has chemopreventive as well as chemotherapeutic effects against various types of cancers. Gong *et al.* [65] showed that CurNPs more efficiently inhibited angiogenesis in a transgenic zebrafish model than free curcumin and the same formulations of CurNPs inhibited the growth of subcutaneous LL/2 pulmonary carcinoma in. Administration of CurNPs (12.5 mg/ kg b.w) for 3 weeks significantly reduced tumor growth in comparison with free curcumin in tumor bearing mice [66]. In the same concern, Zanotto-Filho *et al*. [67] showed that CurNPs at a dose of 1.5 mg/kg/day i.p for 14 days, decreased tumor growth as well as the incidence of intratumoral hemorrhages, necrosis and lymphocytic infiltration. Weekly oral administration of 20 mg/kg b.w CurNPs for 16 weeks succeeded to induce apoptosis in cancer cells in diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) rats [68]. Furthermore, according to the same authors, CurNPs was able to prevent mitochondrial ROS generation, reduce membrane fluidity, increase antioxidant enzyme levels (SOD, CAT) and GSH in hepatic tissues as well as decreased the formation of hyperplastic nodules and atypical nuclei in rats indicating a hepatoprotective effect. These protective effects of CurNPs were mainly due to the small particle size of CurNPs which might be the reason to encourage the circulation kinetics and ability to target the liver tumor(s). Another suggested mechanism for the protection effect of CurNPs against the estrogenic property of ZEN in the current study may be due to the estrogen mimicking activity of CurNPs [69]. Since CurNPs were found to have various effects on the reproductive system of female and male mice such as uterine hypertrophy, reduction of follicle development, hustling of the puberty for female mice and diminishing of accessory sex gland weights, testicular testosterone concentrations and spermatogenesis of male mice.

CONCLUSION

It could be concluded from the current study that ZEN induced severe hapatotoxicity in rat model as indicated by the significant changes in different biochemical, cytogenetic parameters tested and the histological examination of the liver sections. CurNPs could overcome the problems associated to the poor solubility of curcumin and succeeded to induce a potential protection against ZEN mycotoxin and its estrogenic property in male rats. Moreover, CurNPs at the two tested doses were safe and may be promise candidate for the application of curcumin in the drug delivery and food industries application.

Conflict of Interest: The authors declare that we have no financial or non-financial competing interests.

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