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Research Article
Nanoparticles Ellagic Acid Protects Against Cisplatin-induced Hepatotoxicity in Rats Without Inhibiting its Cytotoxic Activity

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Abstract

Background and Objective: Ellagic acid (ELL) is a phenolic constituent of natural sources with antioxidant and anti-inflammatory activities. The ELL has a low bio-availability, therefore a nanoparticles (NP ELL) was formulated to determine whether NP ELL could alleviate cisplatin (CISP)-induced hepatotoxicity in comparison to a high dose of regular ELL. **Materials and Methods:** The NP ELL was prepared using an emulsion solvent diffusion technique. Hepatotoxicity was induced in rats using CISP (7.5 mg kg^{-1}). Rats were pre-treated with either 10 ELL (10 mg kg^{-1}), 1 NP ELL (1 mg kg^{-1}) or 2 NP ELL (2 mg kg^{-1}). **Results:** All ELL regimens significantly decreased CISP-induced increases in liver enzymes, MDA, NO, TNF-α, NFkB and BAX. Additionally, all significantly restored hepatic GSH, GPx, CAT and Bcl-2. Rat's liver showed no injury in all ELL pretreated groups. Importantly, NP ELL did not inhibit the cytotoxic effect CISP against HCT116 cell lines. **Conclusion:** The NP ELL has improved bio-availability and protected against CISP induced hepatotoxicity. This provides an encouraging way of ameliorating the bio-availability of ELL while sustaining its desirable therapeutic effects.

INTRODUCTION

Cisplatin (CISP) is cytotoxic agent that is used in the treatment of solid tumors, such as: Ovarian, bladder and testicular tumors[1,2]. However, its known nephrotoxicity, hepatotoxicity and neurotoxicity have hampered its clinical use in oncology[3-5]. Hepatotoxicity occurs at high doses due to the accumulation of cisplatin in liver cells, this results in inflammation, overproduction of free radicals and a decrease in the antioxidant enzymes[6,7]. The generated oxidative stress appears to be an important mechanism in liver dysfunction[8]. Studies have shown that CISP increases lipid peroxidation and decreases glutathione (GSH) levels in hepatic tissues[9,10]. It also elevates levels of hydroxyl ($^-$OH), peroxynitrite, superoxide-anion (O_2^-) and hydrogen-peroxide radicals[11,12]. CISP-triggered hepatic immune events are indicated by increased tumor necrosis factor-α (TNF-α) and decreased interleukin-10 (IL-10)[13]. During CISP-induced toxicity, levels of Bax (a pro-apoptotic protein) have been shown to increase, while levels of Bcl-2 (an anti-apoptotic protein) have been shown to decrease[13].

The protective effects of several anti-inflammatory agents and anti-oxidants against CISP-induced hepatotoxicity in experimental animals have been investigated[10,14]. Of interest are flavonoids-polyphenolic antioxidants that are found in many vegetables and fruits[15-17]. One such flavonoid, ellagic acid (ELL) is a phenolic constituent in green tea, walnuts and fruits such as grapes, berries and pomegranates. It possesses a variety of biological properties[18] including antioxidant[19-21] and anti-inflammatory[22-24] activities. Researchers have shown that in rats' livers, ELL attenuates cisplatin-enhanced lipid peroxidation and raises GPx and CAT levels. The ELL counteracts the detrimental effects[25] of $^-$OH and O_2^-. In addition, ELL has been found to decrease the level of TNF-α in the livers of rats with lipopolysaccharide-induced sepsis[26]. When combined with p-coumaric acid, ELL also increases cytokine Il-10 (an anti-inflammatory cytokine) in rats' livers in the same sepsis model[26].

The ELL has been also found to be useful in the retraction of several types of tumors including those in lung[27], colorectal[28], esophageal[29] and breast cancers[30]. However, despite its promising properties, ELL has low solubility in water (<10 mg mL^{-1} in phosphate buffer pH) and permeability (0.13×10^{-6}). In addition, it is unstable at the physiological pH[31] of 7.4. When administered orally, it is poorly absorbed and metabolized in the gastrointestinal tract and is thus rapidly eliminated[32,33]. All of which limits the therapeutic benefits of ELL and its clinical uses[34].

The utilization of nanotechnology in the field of alternative medicine is one of the fastest growing fields. The use of nanotechnology has many advantages that have led to a surge in the use of alternative medicine in the treatment of many tumors and chronic conditions. The loading of natural products on nano-particles increases their bio-availability, targeting and controlled-release pattern[35]. Therefore, this study was designed to: (1) Prepare ELL nanoparticles (ELL NP), (2) Determine the protective effects that both regular ELL and NP ELL formulations have on CISP-induced hepatotoxicity in rats, (3) Emphasis the possible protective mechanism of ELL against CISP-induced hepatotoxicity, (4) Examine the effect NP ELL treatment on the cytotoxic activity of CISP using human colorectal adenocarcinoma cells (HCT116).

MATERIALS AND METHODS

Synthesis of nanoparticles ellagic acid (NP ELL): In 10 mL ethanol mix 25 mg regular ELL with 150 mg of lecithin, 100 mg of stearic acid, 80 mg of cholesterol, 20 mg of DSPE-PEG. The mixture was then mixed with a solution containing 5 mL of 1% tween 80 and 5 mL of aqueous solution containing 25 mg of chitosan and was stirred for 40 min at 70°C. After that 40 mL of DI water was added and stirring for about 1 h at 70°C to evaporate the ethanol. The dialyzed product was lyophilized using 5% sucrose.

Particle size determination: A Malvern zetasizer (Malvern Instrumentation Co., Westborough, MA, USA) was used to determine the size distribution of the NP ELL in aqueous dispersions (Fig. 1).

Entrapment/loading efficiency: The quantity of ELL in the nanoparticles was determined by comparing the absorbance at 270 nm to standard curves for different ELL concentrations. The Eq. 1 was used to determine entrapment efficiency:

$$\text{Entrapment efficiency (loading)} = \frac{(\text{Drug})f}{(\text{Drug})t} \times 100 \qquad (1)$$

where, (Drug)f represents the the concentration of ELL in the nanoparticles. (Drug)t represents the theoretical concentration of ELL.

The entrapment efficiency was found to be around 55%. The overall loading of ELL in nanoparticles were found be around 0.84% w/w (Fig. 2).

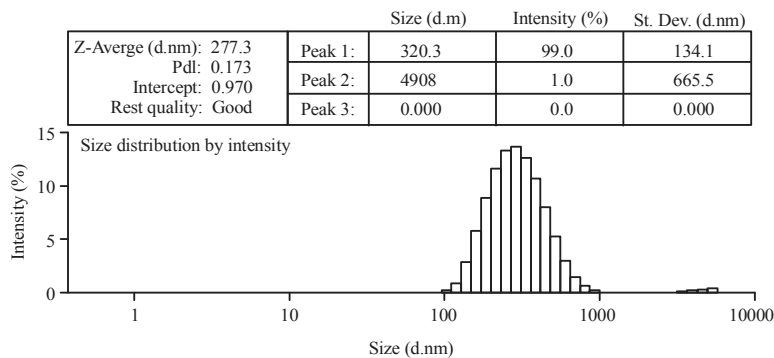|		Size (d.m)	Intensity (%)	St. Dev. (d.nm)
Z-Averge (d.nm): 277.3	Peak 1:	320.3	99.0	134.1
Pdl: 0.173	Peak 2:	4908	1.0	665.5
Intercept: 0.970 Rest quality: Good	Peak 3:	0.000	0.0	0.000

Fig. 1: Size measurement of nanoparticles ellagic acid (NP ELL) using dynamic light scattering (DLS). Average particle size is around 277 nm in diameter, PDI = 0.173

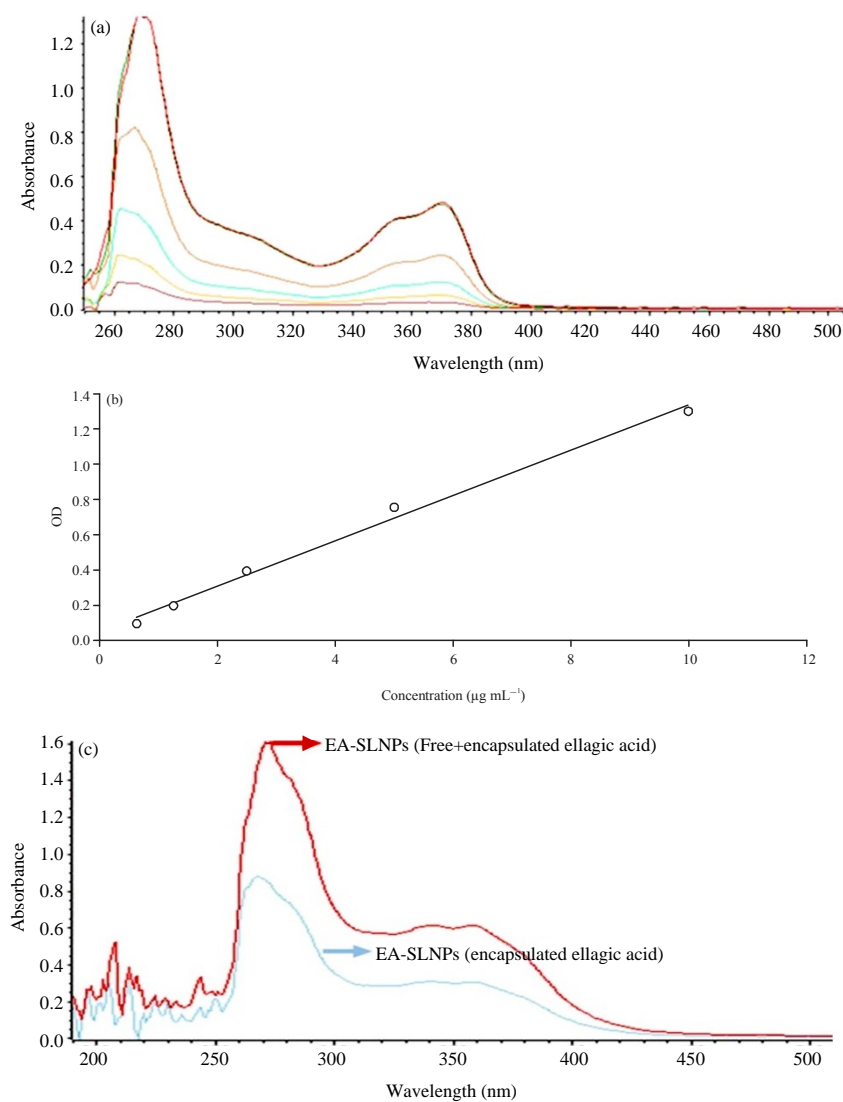Fig. 2(a-c): Determination of entrapment/loading efficiency of encapsulated nanoparticles ellagic acid (NP ELL), (a) UV-VIS spectra used to construct the standard curve of ELL, (b) With concentrations of PE from 0.625, 1.25, 2.5, 5 and 10 μg mL^{-1} and (c) Determination of entrapment efficiency by comparing OD from UV-Vis spectra of total amount ELL (free+encapsulated) and encapsulated ELL in the SLNPs

Animals: Thirty male Sprague Dawley rats (150-180 g) were separated into five groups (n = 6). Control group (CONT): received no treatment, cisplatin group (CISP): Received a single-dose of CISP (7.5 mg kg^{-1}) intraperitoneally (i.p.)[36], regular ellagic acid group (10 E LL): Received a single dose of regular ellagic acid (10 mg kg^{-1})[10], 6 h before CISP, 1st nanoparticle ellagic acid group (1 NP ELL): Received single dose of NP ELL (1 mg kg^{-1}), 6 h before CISP, 2nd nanoparticle ellagic acid group (2 NP ELL): Received a single dose of NP ELL (2 mg kg^{-1}), 6 h before CISP. The study protocol was approved (No. 1438-108) by the Scientific Research Ethics Committee of the Faculty of Pharmacy, KAU, KSA.

Sample collection: About 24 h after CISP injection rats were anesthetized with ether and blood samples were withdrawn. Serum was then separated and kept at -80°C. Rats were then killed by cervical dislocation and the livers were extracted. Parts of the livers were preserved in 10% neutral formalin solution while the remaining parts of the liver were kept at -80°C.

Liver functions measurement: Detection kits (Human, Germany) were used to determine the liver enzymes aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) according to the manufacturing instructions.

Oxidative stress markers and antioxidants enzymes measurement: Kits purchased from Abcam (USA) were used to determine the levels of malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) in the liver homogenates based on the manufacturer's instructions.

ELISA measurements: Nuclear factor kappa beta (NFkB), interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α) levels were measured in the liver homogenates using ELISA kits of Abcam (USA) for NFkB, R and D Systems, Inc., kit., (USA) for TNF-α and Novex kit (USA) for IL-10. The kits use primary antibody raised against rat NFkB, IL-10 and TNF-α.

Hematoxylin and eosin staining: Hematoxylin and eosin (H and E) were used to stain 4 µm paraffin-embedded livers, which were then examined and photographed utilizing the light microscope (Nikon Eclipse TE2000-U, NIKON, Japan).

Immunohistochemical staining: Liver sections were stained utilizing an immunoperoxidase (PAP, peroxidase/ anti-peroxidase) technique using Lab Vision (Fremont, CA) NF-kB, BAX and Bcl-2 antibodies. The slides were filmed using light microscopy (Nikon Eclipse TE2000-U, NIKON, Japan) and quantified by Image J software (Image J, 1.46a, NIH, USA).

Cell culture: The HCT116 was obtained from the Vaccera (Giza, Egypt). The HCT116 cells were cultured in RPMI-1640 media containing streptomycin, penicillin and fetal bovine serum (10%). Cells were incubated at 37°C in a humidified incubator with 5% CO_2.

***In vitro* SRB cytotoxicity assays:** The cytotoxicity was tested using sulforhodamine B (SRB) assay as previously described[37]. Breifly, HCT116 cells were plated in 96-well plates. Serial concentrations of CISP alone or in combination with NP ELL (1 and 10 µM) was applied to the cells. Cells were incubated for 72 h and then SRB (0.4%) was added and the cells were incubated for another 10 min. The plate was then kept drying overnight and the SRB-stained cells were dissolved using Tris-HCl. About 540 nm wavelength was used to determine color intensity. The E_{max} model was used to analyze the dose response curve of each compound[38].

Statistical analysis: Results were represented as mean±standard error (SEM). Values were statistically analyzed utilizing one-way analysis of variance (ANOVA) followed by Tukey-Kramer test (package Minitab 18, Minitab. Inc., State College PA, USA). The level of significance was settled at $p \leq 0.05$.

RESULTS

Serum liver function enzymes (AST, ALT and ALP): Animals treated with CISP (7.5 mg kg^{-1}) showed significant increase in serum AST, ALT and ALP levels compared to the CONT group (Fig. 3a-c). Animals pretreated with 10 mg kg^{-1} regular ELL, 1 mg kg^{-1} NP ELL and 2 mg kg^{-1} NP ELL showed significant decrease in serum AST, ALT and ALP levels compared to the CISP group (Fig. 3a-c).

Liver oxidative stress markers (MDA, NO and GSH): Animals treated with CISP showed significant increase in liver MDA and NO contents compared to the CONT group (Fig. 4a and b). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant decrease in liver MDA and NO contents compared to the CISP group (Fig. 4a and b).

On the other hand, animals treated with CISP showed significant decrease in liver GSH content compared to the CONT group (Fig. 4c). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant increase in liver GSH content compared to the CISP group (Fig. 4c).
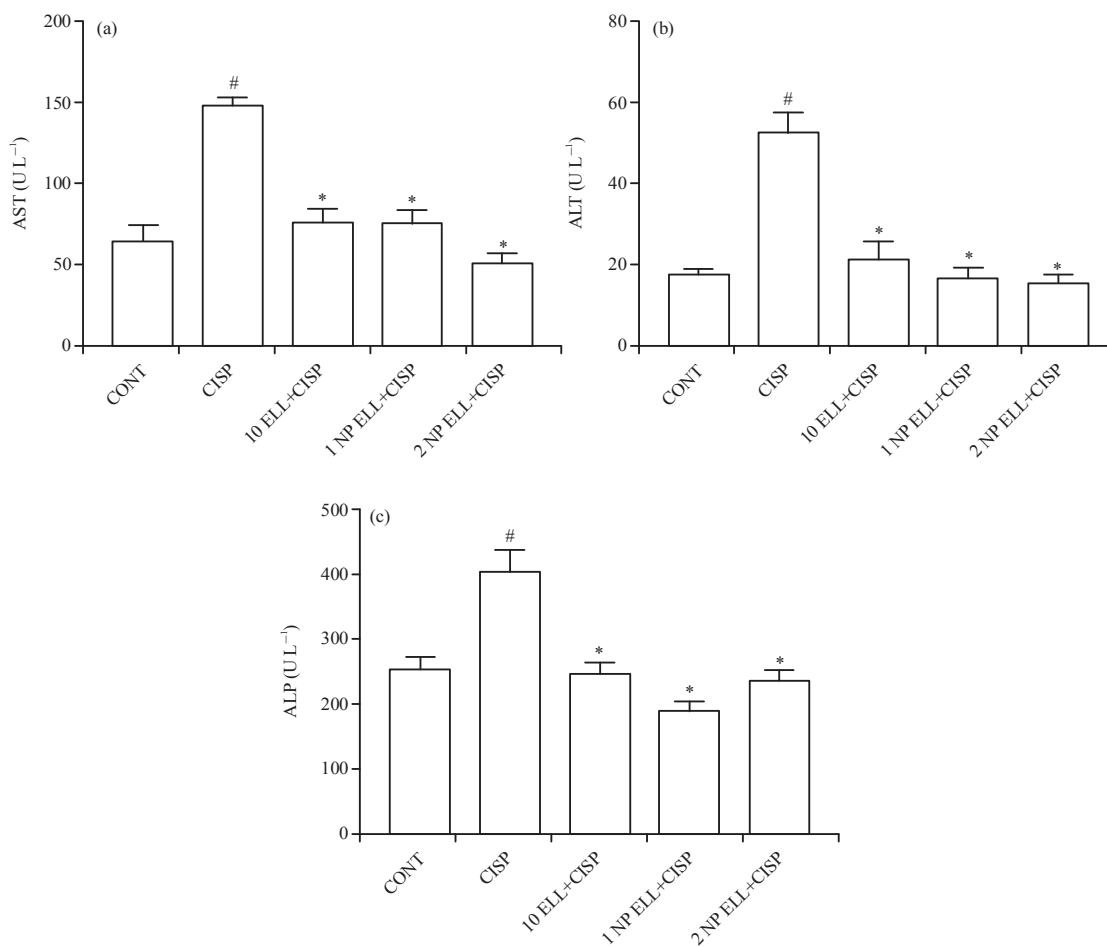
Fig. 3(a-c): Levels of serum (a) AST, (b) ALT and (c) ALP measured in control (CONT), cisplatin (CISP, 7.5 mg kg^{-1}), ellagic acid (10 ELL, 10 mg kg^{-1}), nanoparticles ellagic acid (1 NP Ell, 1 mg kg^{-1}) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg^{-1}). Data are expressed as mean±SEM of 6 rats/group. #p<0.05 compared with the CONT group. *p<0.05 compared with the CISP group
Data are compared using ANOVA followed by Tukey-Kramer *post hoc* test

Fig. 4(a-c): Continue

Fig. 4(a-c): Levels of liver (a) MDA, (b) NO and (c) GSH measured in control (CONT), cisplatin (CISP, 7.5 mg kg^{-1}), ellagic acid (10 ELL, 10 mg kg^{-1}), nanoparticles ellagic acid (1 NP Ell, 1 mg kg^{-1}) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg^{-1}). Data are expressed as mean±SEM of 6 rats/group. #$p<0.05$ compared with the CONT group. *$p<0.05$ compared with the CISP group

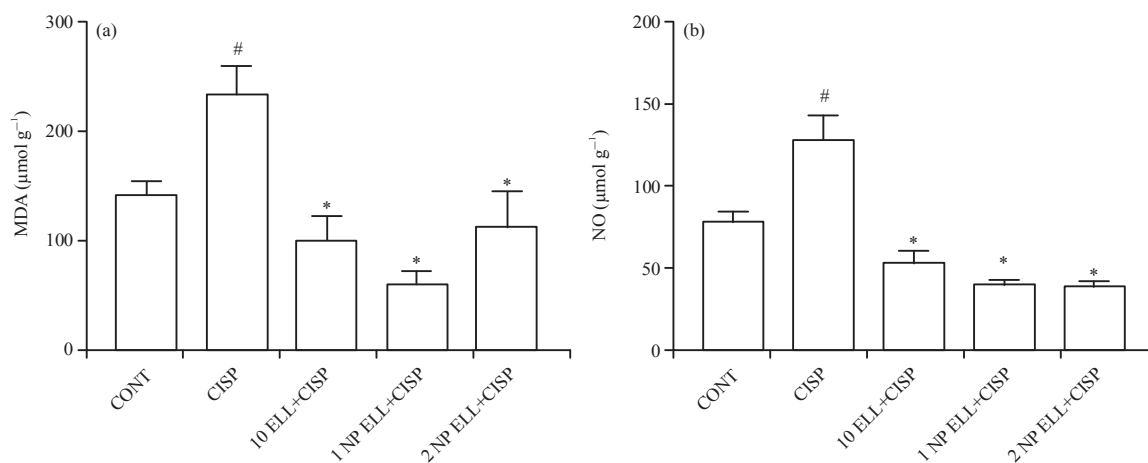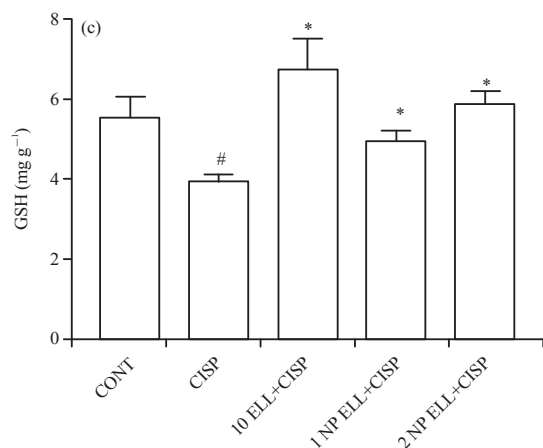Data are compared using ANOVA followed by Tukey-Kramer *post hoc* test

Fig. 5(a-b): Levels of liver (a) Gpx and (c) CAT measured in control (CONT), cisplatin (CISP, 7.5 mg kg^{-1}), ellagic acid (10 ELL, 10 mg kg^{-1}), nanoparticles ellagic acid (1 NP Ell, 1 mg kg^{-1}) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg^{-1}). Data are expressed as mean±SEM of 6 rats/group. #$p<0.05$ compared with the CONT group. *$p<0.05$ compared with the CISP group

Data are compared using ANOVA followed by Tukey-Kramer *post hoc* test

Liver antioxidant enzymes (GPx and CAT): Animals treated with CISP showed significant decrease in liver GPx and CAT activities compared to the CONT group (Fig. 5a and b). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant increase in liver GPx and CAT activities compared to the CISP group (Fig. 5a and b).

Liver cytokines (IL-10 and TNF-α): Animals treated with CISP showed significant decrease in liver IL-10 content compared to the CONT group (Fig. 6a). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant increase in liver IL-10 content compared to the CISP group (Fig. 6a).

On the other hand, animals treated with CISP showed significant increase in liver TNF-α content compared to the CONT group (Fig. 6b). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant decrease in liver TNF-α content compared to the CISP group (Fig. 6b).

Liver NFkB: Animals treated with CISP showed significant increase in liver NFkB content compared to the CONT group (Fig. 6c). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant decrease in liver NFkB content compared to the CISP group (Fig. 6c).
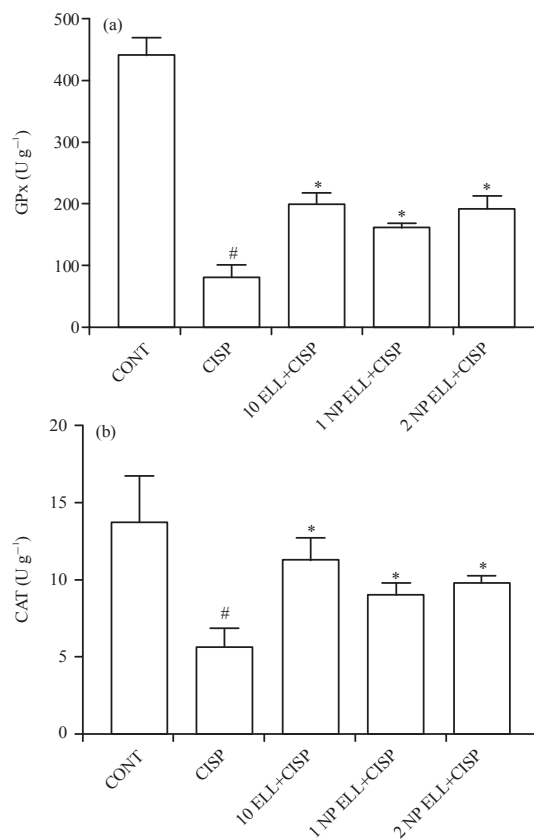
In addition, immunohistochemical expression of NFkB revealed that animals treated with CISP showed significant increase in liver NFkB expression compared to the CONT group (Fig. 7a and d). Animals pre-treated with ELL, 1 NP ELL and 2 NP ELL showed significant decrease in liver NFkB expression compared to the CISP group (Fig. 7a and d).

Liver apoptotic markers (BAX and Bcl-2): Immunohistochemical expression of BAX revealed that animals treated with CISP showed significant increase in liver BAX expression compared to the CONT group (Fig. 7b and e). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant decrease in liver BAX expression compared to the CISP group (Fig. 7b and e).
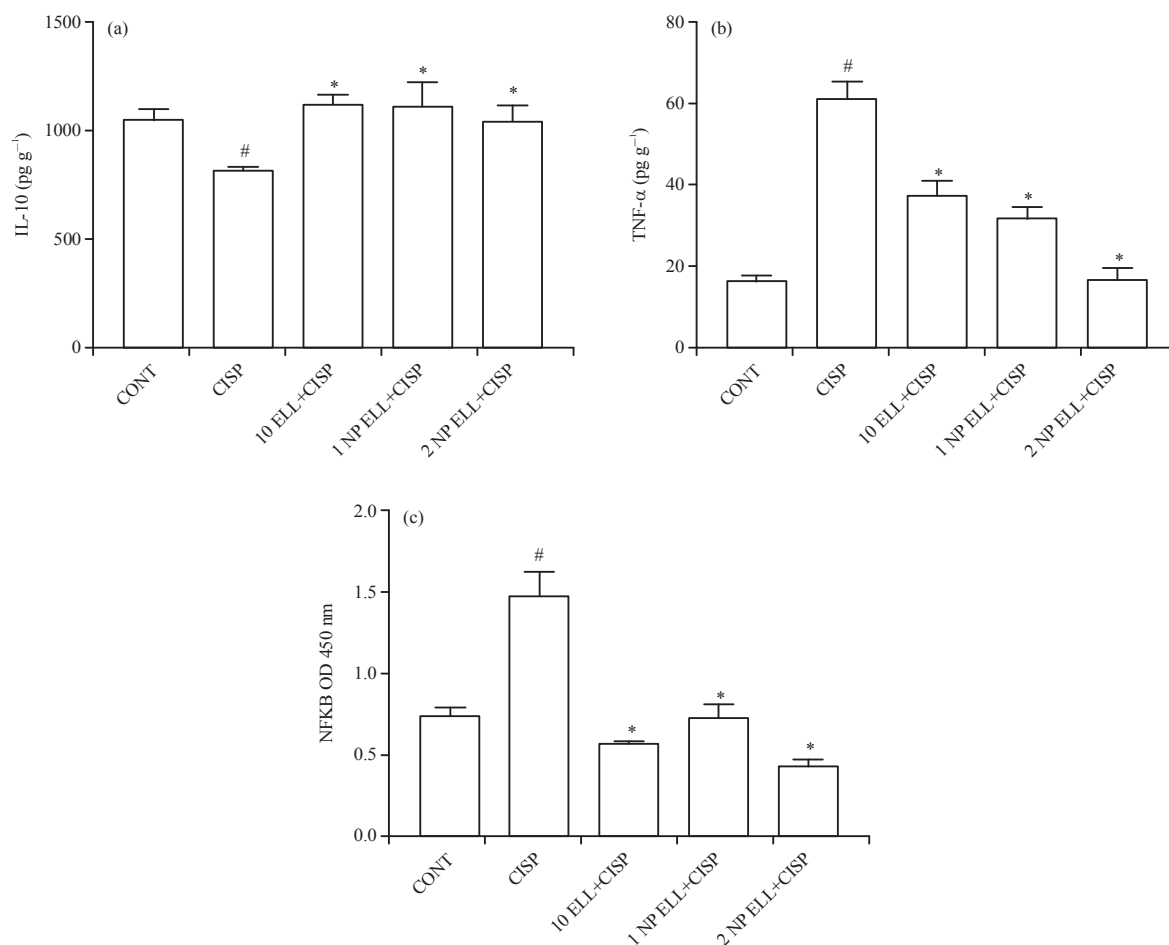
Fig. 6(a-c): Levels of liver IL-10 (a), TNF-α (b) and NfkB (c) measured in control (CONT), cisplatin (CISP, 7.5 mg kg⁻¹), ellagic acid (10 ELL, 10 mg kg⁻¹), nanoparticles ellagic acid (1 NP Ell, 1 mg kg⁻¹) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg⁻¹). Data are expressed as mean$\pm$SEM of 6 rats/group. #$p<0.05$ compared with the CONT group. *$p<0.05$ compared with the CISP group
Data are compared using ANOVA followed by Tukey-Kramer *post hoc* test

On the other hand, immunohistochemical expression of Bcl-2 revealed that animals treated with CISP showed significant decrease in liver Bcl-2 expression compared to the CONT group (Fig. 7c and f). Animals pretreated with ELL, 1 NP ELL and 2 NP ELL showed significant increase in liver Bcl-2 expression as compared to the CISP group (Fig. 7c and f).

Liver H and E histopathology: In Fig. 8a and b, control animals showed normal hepatocytes and blood sinusoids. In Fig. 8c and d, CISP treated animals showed swollen hepatocytes with compressing blood sinusoids, hydropic degeneration and pyknotic nuclei. In Fig. 8e and f, Ell treated animals showed normal hepatocytes, dilated sinusoids and few hepatocytes still showed dark pyknotic nuclei. In Fig. 8g and h, 1 NP ELL treated animals showed marked protection where most hepatocytes showed intact outlines, rounded

vesicular active nuclei and stained cytoplasm. In Fig. 8i and j, 2 NP ELL treated animals showed that most hepatocytes are of well-defined outlines, stained cytoplasm and large vesicular active nuclei.

Anticancer activity of CISP: Pre-treatment with 1 or 10 μM NP ELL non-significantly increased the IC_{50} of CISP as a single agent from 1.4-1.6 μM and 2.0 μM, respectively, confirming that NP ELL did not inhibit CISP cytotoxicity (Fig. 9a and b).

DISCUSSION

Nanotechnology is a useful tool for the amelioration of the bioavailability of natural products. This allows us to improve their effectiveness in the prevention and treatment of many diseases. Since many natural products are highly
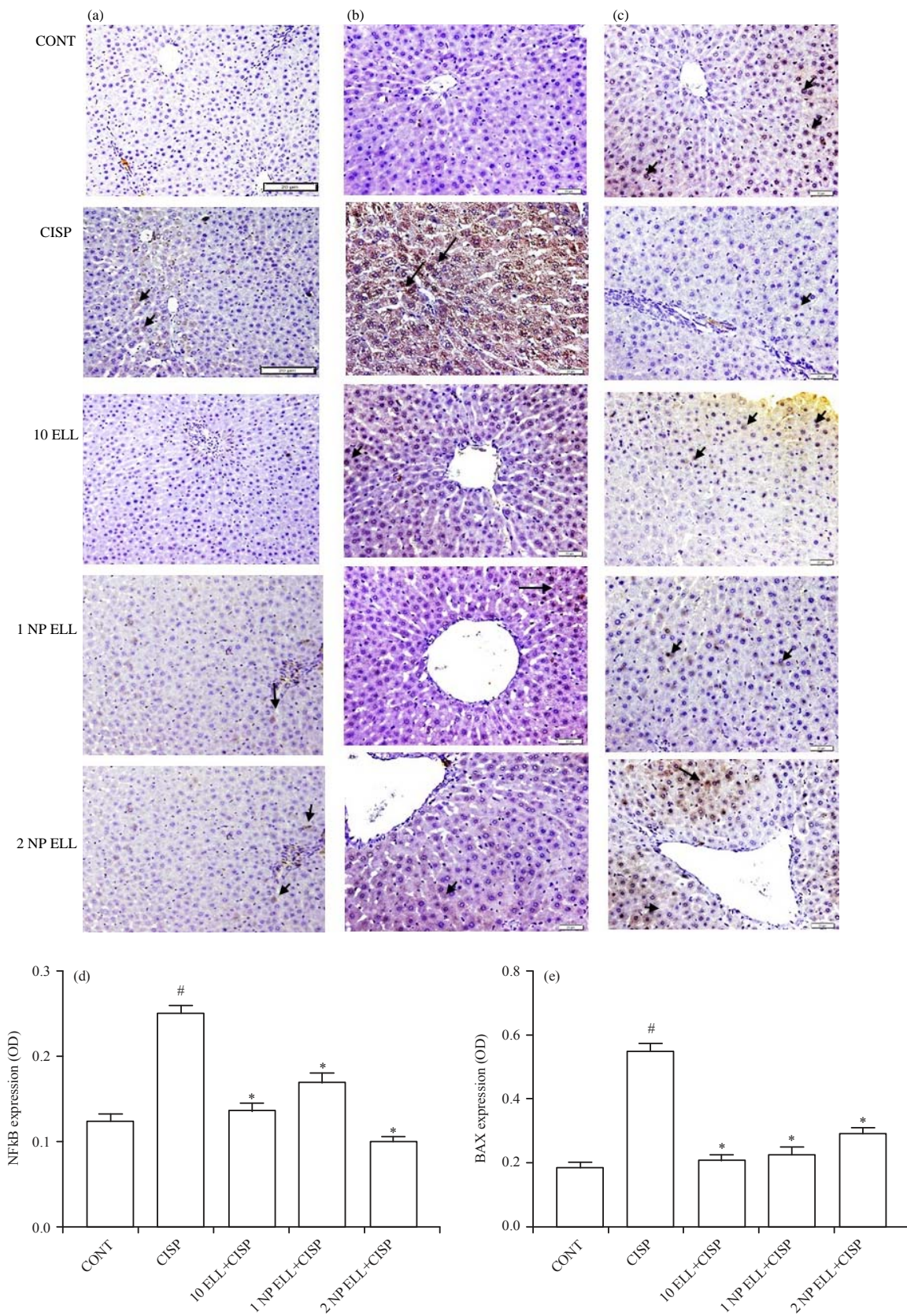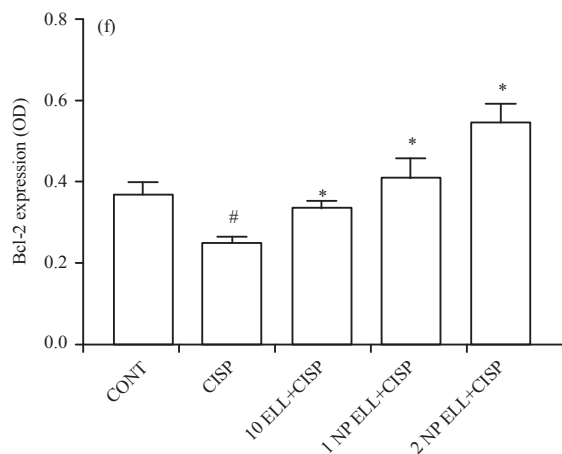
Fig. 7(a-f): Continue

lipophilic, they are not suitable for drug delivery because they do not dissolve well in the blood. Because of the lack of bioavailability of these products, administration of high doses is usually required to produce a therapeutic response. The use of these compounds in such a high dose can lead to symptoms of acute toxicity as well as the inconsistency of patients use. Only converting these lipophilic products into nanoparticles can increase their solubility and efficacy[35]. Although ELL has promising therapeutic potential, it is poorly soluble and has low permeability and stability at physiological pH[31]. In order to overcome this problem preparing ELL in nanoparticles may be a fruitful way to improve its biopharmaceutical properties and render it more clinically useful. Furthermore, nano-encapsulation of ellagic acid was shown to be effective in preventing cyclosporine-A nephrotoxicity at a three-fold lower concentration[39]. The focus of the current study was whether a very low dose (1 and 2 mg) of NP ELL can alleviate CISP-induced hepatotoxicity compared to a high dose (10 mg) of regular ELL.

The current study, highlighted that NP ELL is as effective or in some instances superior to ELL in protecting against CISP induced hepatotoxicity in rats. Administration ELL, 1 NP ELL and 2 NP ELL effectively restored: i) Serum levels of AST, ALT and ALP, ii) Liver levels of MDA, NO, GSH, GPx, CAT, IL-10, TNF-α and NFkB. Furthermore, immunohistochemical examination revealed that the administration of ELL, 1 NP ELL and 2 NP ELL restored the CISP-induced alteration of liver expression of
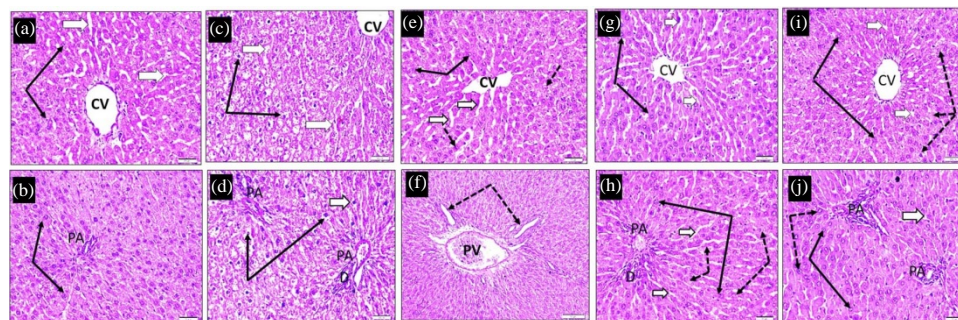
Fig. 7(a-f): Immunohistochemistry photomicrographs of liver sections showing the effect of ellagic acid (10 ELL, 10 mg kg^{-1}), nanoparticles ellagic acid (1 NP Ell, 1 mg kg^{-1}) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg^{-1}) on cisplatin (CISP)-induced alteration of liver expression of NFkB (a), BAX (b) and Bcl-2 (c). The bar chart d, e and f show liver NFkB, BAX and Bcl-2 OD in the different experimental groups. Data are expressed as Mean±SEM of 6 rats/group. #p<0.05 compared with the CONT group. *p<0.05 compared with the CISP group

Data are compared using ANOVA followed by Tukey-Kramer *post hoc* test

Fig. 8(a-j): Liver cross sections (H and E stain x 400) obtained from control (CONT) (a and b), cisplatin (CISP, 7.5 mg kg^{-1}) (c and d), ellagic acid (10 ELL, 10 mg kg^{-1}), nanoparticles ellagic acid (1 NP Ell, 1 mg kg^{-1}) (e and f) and nanoparticles ellagic acid (2 NP Ell, 2 mg kg^{-1}) (g and h) showing hepatocytes at Central vein (CV) and portal area (PA) regions. a and b CONT: hepatocytes are arranged in cell plates they have acidophilic cytoplasm and large central vesicular active nuclei (thin black arrows). Blood sinusoids between cell plates are normal and lined by endothelial cells (white arrows). c and d CISP: hepatocytes are swollen compressing blood sinusoids (white arrows), their cytoplasm is unstained (hydropic degeneration) and their nuclei (thin black arrows) are small dark stained and degenerated (pyknotic). e and f 10 Ell: showing normal hepatocytes (black arrows), dilated sinusoids (white arrows). Few hepatocytes still showed dark pyknotic nuclei or vacuolated cytoplasm (dotted arrows). g and h 1 NP Ell: Showing marked protection where most hepatocytes showed intact outlines, rounded vesicular active nuclei and stained cytoplasm (thin black arrows). Few scattered cells showed ill-defined outlines, slightly vacuolated cytoplasm and small or absence of nuclei (dotted arrows). i and j 2 NP Ell: Most hepatocytes showed well defined outlines, stained cytoplasm and large vesicular active nuclei (thin black arrows)
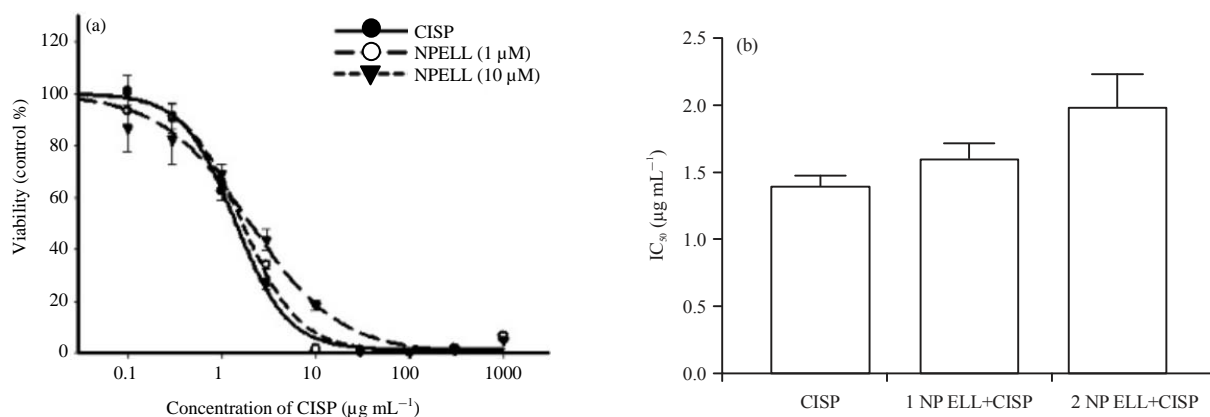
Fig. 9(a-b): Cytotoxicity of CISP was assessed *in vitro* with and without NP ELL against HCT 116 colorectal cancer cells (a) CISP, CISP+NP ELL (1 μM) and CISP+NP ELL (10 μM) using SRB viability assay and (b) Calculated IC_{50} of CISP in presence of NP ELL (1 and 10 μM) in HCT116 cells, respectively. Data are expressed as mean±SEM of 6 rats/group. *p<0.05 compared with the CISP group
Data are compared using ANOVA followed by Tukey-Kramer post hoc test

NFkB, BAX and Bcl-2 to pre-treatment levels. Examination of liver sections showed that ELL, 2 NP ELL and 2 NP ELL resulted in a marked protective effect on hepatocytes in comparison to the CISP group, with 1 NP ELL and 2 NP ELL showing a higher protective effect.

The findings in this study agree with Yuce *et al.*[10] in which ELL prevented CISP induced liver toxicity. Yuce *et al.*[10] reported that regular formulation of ELL restored levels of MDA, GSH, GSH-Px and CAT as well as ameliorated the histopathological changes caused by CISP on liver and heart tissue in rats[10]. Restoration of serum levels of AST, ALT and ALP in the current study further supports the protection of ELL in its various formulations against CISP induced hepatotoxicity. Liver cells contain high levels of hepatic enzymes. Increased levels of liver enzymes in the serum are a strong sign for cell membrane function loss and even hepatic cell damage. The CISP toxicity leads to liver cell damage and leakage of AST, ALT and ALP enzymes into the blood stream[40].

The CISP is a generator of reactive oxygen species and its administration results in an up regulation of free radicals, which ultimately lead to the destruction of cellular structures[1,41]. It has been postulated that higher levels of MDA found in the CISP treated animals as found in the present study, is due to a reduction of anti-oxidants in these tissues[36]. GSH is a key-player in cellular metabolism and possesses reducing properties, which are essential in preserving the integrity of cells[41]. The CAT and GPx have a protective role against reactive oxygen species. Therefore, the restoration of CAT and GPx is key to the hepatoprotection caused by ELL in its various formulation. This restoration of anti-oxidants was consistent with other studies, which examined the role that anti-oxidants had in relation to CISP toxicity in animals[36,42].

These results are concurrent with the study by Yuce *et al.*[10]. They reported an elevation of hepatic markers of oxidative stress in CISP treated rats, with a protective antioxidant effect exerted by ELL[10]. The ELL possesses a strong ability to remove free radicals both *in vivo* and *in vitro*[43,44].

The roles of TNF-α and IL-10 have been well recognized in linking three different mechanisms, which underlie acute toxicity cases: Inflammation, oxidative stress and apoptosis[45-47]. CISP toxicity caused an enormous inflammatory reaction in liver cells, which is confirmed by elevated blood level of TNF-α and other anti-inflammatory mediators as well as decreased level of IL-10[45,48]. The TNF-α supported the liver tissue inflammation through increasing the chemotaxis of immune cells and activation of other inflammatory cytokines[49]. Moreover, the fall in IL-10 exacerbates liver damage because it down regulates antigen presentation and the pro-inflammatory cytokines liberation[50,51]. Liver levels of IL-10 and TNF-α were also restored by ELL in the present study further supporting its hepatoprotective role. The 10 ELL, 1 NP ELL and 2 NP ELL showed a marked protective effect to CISP-induced hepatotoxicity in rats. This was mainly attributed to the down regulation of TNF-α and increased levels of IL-10. Furthermore, it also acted by indirectly by inhibiting ROS liberation. These effects collectively supported the use of 10 ELL, 1 NP ELL and 2 NP ELL in treating CISP-induced liver complications.

Similar to a number of other reports, this study found that the expression of NFkB was up regulated in CIS treated animals[42,52,53]. In addition, the current data showed that, 10 ELL, 1 NP ELL and 2 NP ELL pre-treatment decreased NF-kB expression in the liver of CISP-treated rats[54]. It is well known that NFkB plays a central role in regulating inflammatory

mediators and its activation results in apoptosis[53,55]. The NF-kB is a cytoplasmic nuclear transcription factor bound to I-kB (an inhibitory subunit). In the presence of excess reactive oxygen species, NFkB is activated and dissociated from I-kB. Consequently, NF-kB moves to the nucleus of the hepatocyte binds to DNA and enhances the transcription of plenty of the inflammatory genes like cytokine, chemokine which are involved in inflammatory and immune responses and the predisposition to fibrosis, apoptosis and acute phase responses that cause tissue and organ damage[56].

The BAX and Bcl-2, which are pro and anti-apoptotic respectively were shown to play a role in CISP-induced cell death[57]. In the current study, BAX was increased by CIS, whereas Bcl-2 levels were decreased which is consistent with previous studies[36,42]. This effect was reversed in the presence of ELL.

The cytotoxicity of CISP in the presence of NP ELL was assessed using HCT116 colorectal cancer cell. The results confirmed that both 1 NP ELL and 2 NP ELL did not inhibit the cytotoxic effect CISP against HCT116 colorectal cancer cells.

CONCLUSION

Nanoformulation of ELL improves its bio-availability and protects against CISP induced hepatotoxicity. This provides an encouraging way of ameliorating the bio-availability of ELL while sustaining its desirable therapeutic effects.

SIGNIFICANCE STATEMENT

The results of this study showed for the first time that two very low doses of NP ELL are very effective in mitigating CISP-induced hepatotoxicity. The effect is comparable to that of a high dose of regular ELL. This finding will be beneficial to many patients suffering from various cancers treated with CISP. In addition, it opens the field in front of researchers to search the protective effect of the NP ELL in human beings.

ACKNOWLEDGMENT

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