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Ameliorative Effects of Melittin Nanoparticles Against Zearalenone Induced Hepatotoxicity in Female Rats

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Abstract

Background: Nanoparticle's formulation of natural products is recently posed effective medicinal applications against different clinical disorders. Zearalenone (ZEA) is certainly considered one among mycotoxins which might be from corn, sorghum and wheat. In the liver, ZEA is metabolized into α- and β-zearalenol, which are considered more toxic than ZEA. The present study investigated biochemical and histological ameliorative effects of Melittin (MEL) and MEL-loaded chitosan nanoparticles (Cs-NPs), a primary polypeptide in the venom of honeybee (Apis mellifera) against hepatotoxicity induced by ZEA. The ionic gelation process was used to make MEL-loaded chitosan-TPP nanoparticles that contained chitosan, MEL, and TPP salt. Rats were divided into 4 groups. Control: given orally 2.7 mg/kg b.w. of 1% DMSO saline, T1: given orally 2.7 mg/kg b.w. of ZEA, T2: given orally 2.7 mg/kg b.w. of ZEA, then given orally MEL 40 µg/kg b.w after intoxication. T3: given orally 2.7 mg/kg b.w. of ZEA, then given orally MEL loaded on Cs-NPs 40 µg/kg b.w after intoxication. DMSO saline and ZEA were given to rats twice a week for two weeks, while MEL/MEL nanoparticles were given three-time weekly for one month after ZEA intoxication, Serum ALT, AST, ALP, Albumin, Total protein, alpha-fetoprotein, TNF-α, and total antioxidant status was assessed. Histological changes in the rat's liver were matched with biochemical changes.

Results: FT-IR indicated that MEL was successfully loaded into CS-NPs. TEM with average diameter of 37.5 nm and a spherical shape for MEL-loaded chitosan-TPP nanoparticles. The encapsulation efficiency (EE%) of MEL in chitosan-TPP nanoparticles was 82.35%. our study demonstrated that MEL-loaded chitosan-TPP nanoparticles confer ameliorative effects against ZEA-induced hepatic biochemical and histological alteration.

Conclusion: The present study demonstrated that nanoparticles like chitosan-tripolyphosphate (TPP) can increase the antihepatotoxic properties of MEL and can be utilized as a potent agent in the treatment of chemical-induced hepatotoxicity.

Keywords: Mycotoxins, Zearalenone, Hepatotoxicity, Chitosan-TPP, MEL, Nanoparticles.

1. Introduction

Fusarium species produce zearalenone (ZEA), a macrocyclic-resorcylic acid lactone [1]. It's one of the most frequent mycotoxins, found in contaminated agricultural and food products such maize, wheat, barley, and oats [2, 3]. Due to its high-affinity binding of the estrogenic receptors, ZEA exhibit potent reproductive toxicity, hepatotoxicity, immunotoxicity, and genotoxicity in humans and animals [4]. Moreover, ZEA is extremely heat-stableand resistant to conventional degradation methods such as physical and chemical approaches [5].

ZEA can affect the development and function of the liver. The shape of Kupffer cells in the liver can be affected by low doses of ZEA [6]. High levels of ZEA in the liver can cause immunosuppression and potentially liver damage [7].

Bee venom therapy has been used for hundreds of years to treat acute and chronic human illnesses [8]. The toxin contains a variety of bioactive peptides and enzymes. Among them, melittin (MEL)

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is a polypeptide constituting about 50% of the dry weight of the toxin [9]. Emerging evidence suggests that the peptide has multiple biological functions including antioxidant, anti-inflammatory, and antibacterial properties [10, 11].

Chitosan is a linear polysaccharide made from crustacean shells and fungal cell walls. It has been used as a highly biocompatible, biodegradable and safe material in drug delivery systems [12, 13]. Nanoparticles are made from a variety of biodegradable materials and are typically less than 500 nm in size [14, 15]. Cs-NPs can increase drug activity more efficiently than chitosan solution [16, 17] Therefore, chitosan-tripolyphosphate (TPP)-nanoparticles have been widely applied to deliver drugs across tissues. Overall, using chitosan-TPP nanoparticles as a nano-system can increase MEL delivery in infected tissue [18, 19]. In the present study, we assessed the ameliorative effects of MEL-loaded Cs-NPs and the peptide, MEL as a major component of bee venom against hepatotoxicity induced by the administration of ZEA in female rats.

2. Experimental

2.1. Chemicals

ZEA fungal mycotoxin purchased from Sigma Aldrich, Egypt – CAS Number: 17924-92-4 and the Product Number Code is (Z2125-10 mg). Purity (HPLC) of ZEA ≥ 99.0%, MW: 318.36 g/mol. ZEA was dissolved in DMSO (dimethyl sulfoxide) and diluted to 1:100 in a sterile saline solution (0.9 % NaCl) to produce a working stock solution and stored at - 20 ºC. MEL from honeybee venom purchased from Sigma Aldrich, Egypt – CAS Number: 20449-79-0 and the Product Number Code is (M2272-10 mg). Purity (HPLC) of MEL ≥ 85.0%, MW: 2,846.46 g/mol. MEL was dissolved in distilled water at a concentration 5mg/ml to produce a working stock solution. Chitosan derived from shrimp shells (Pandalus Borealis), was purchased from Oxford Laboratory Chemicals, India, with a deacetylation degree (DD) of 93% and a low molecular weight of 161.16 (monomer). Sodium tripolyphosphate (STPP) was purchased from Sigma Aldrich, Egypt – CAS Number: 7758-29-4 and the Product Number Code is (238503). The purity of STPP is 85.0%.

2.2. *Animals*

Forty-eight female Wistar rats (180−200 g), 10−12 weeks old, were purchased from the Experimental Animal Unit of Sohag University, Sohag, Egypt. Rats were housed in an animal house under an ambient temperature of 25 ± 1 °C with 12 h light/dark cycles, and 55-60% relative humidity after initial acclimatization for about one week before starting the experiment. They had free access to a standard rodent pellet diet and water ad libitum.

2.3. Study design

The animals were distributed randomly into 4 groups, one control and three treated (12 Rats/group), as follows:

Control: Rats were given orally 2.7 mg/kg b.w. of 1% DMSO saline, two doses (Twice a week) for two weeks.

Treated 1: Rats were given orally 2.7 mg/kg b.w. of ZEA, two doses (Twice a week) for two weeks. (Chronic dose), according to [20].

Treated 2: Rats were given orally 2.7 mg/kg b.w. of ZEA, two doses (Twice a week) for two weeks, then given orally MEL 40 µg/kg b.w after intoxication, Three-time weekly for one month, according to [21].

Treated 3: Rats given orally 2.7 mg/kg b.w. of ZEA two doses (Twice a week) for two weeks, then given orally MEL-loaded on Cs-NPs 40 µg/kg b.w after intoxication, Three-time weekly for one month.

Rats were scarified and dissected at the end of each period, and blood samples from all groups were collected from the heart into plain tubes and centrifuged at 4000 rpm for 10 minutes to separate the serum, which was then divided into multiple aliquots and stored at -20°C until the evaluation. Moreover, the whole livers of all groups were collected for histological studies.

2.4. MEL-loaded chitosan-TPP nanoparticles synthesis

The production of nanoparticles using the ionic gelation process is based on electrostatic interactions between negatively and positively charged molecules such as polyanionic and cationic polymers [22]. For MEL-loaded chitosan TPP nanoparticles, the amino group (positive charge) present in chitosan interacts with the anionic group (negative charge) of the TPP salt. The MEL-loaded Cs-NPs were formed according to the method reported by [23].

2.4.1. MEL-loaded chitosan-TPP nanoparticles characterization

The prepared MEL-loaded Cs-NPs were characterized by the following methods:

2.4.1.1. Ultraviolet–visible Spectroscopy (UV-Vis)

To estimate the free amount of drug in the supernatant, the solution was scanned at 280 nm in a spectrophotometer (UviLine 9400) using a quartz cuvette with water as the reference [24].

2.4.1.2. Fourier-Transform Infrared (FT-IR) Analysis

Fourier Transform Infrared Analysis (FT-IR) was performed to confirm the synthesis of nanoparticles and detect possible chemical interactions between

chitosan and MEL [25]. For FT-IR, each sample was scanned from 3500 to 550 cm-1 in FT-IR Spectrophotometer (ATR-FTIR, Alpha Bruker Platinum, 1-211-6353) at a resolution limit of 4 cm−1. FTIR spectra of CS, TPP, MEL, and MEL loaded CS-NPs were obtained by placing a small portion of the sample on the sensor of the instrument, and the spectrum was then compared with the spectrum of chitosan, MEL, and TPP standard.

2.4.1.3. Transmission electron microscopy (TEM)

TEM analysis was applied at NanoTech Company, Egypt. The size and the surface morphology of MEL-Cs-NPs were observed by transmission electron microscopy (TEM) (JEOL JEM 1200 EXII, Netherlands). The sample for transmission electron microscopy (TEM) analysis was tested according to [26].

2.4.1.4. Evaluation of MEL nanoparticles encapsulation efficiency and Loading Capacity of Nanoparticles

The encapsulation efficiency was analyzed according to the procedure reported by Cevher et al [27]. To obtain a nano-system with a maximum ratio of drug loading, The amount of MEL was dissolved in a certain amount of chitosan-TPP nanoparticles. After MEL loading, nanoparticles were separated from the suspension by ultracentrifugation (Hanil Micro 17TR centrifuge - HE5) at 17000 rPm and 4°C for 30 min. The amount of free MEL in the supernatant was measured by UV-spectrophotometer at a wavelength of 280 nm using distilled water as a solvent.

The Encapsulation efficiency (EE) [28] of nanoparticles were calculated using the following equation:

Encapsulation efficiency (%) = [(T - F) / T] × 100

Where, F is the free amount of MEL (Non encapsulated MEL) in the supernatant, and T is the total amount of MEL added to CS solution.

2.5. Kits

Enzyme immunoassay for the quantitative determination of alpha-fetoprotein in serum was purchased from (BIOS Company, South San Francisco, CA 94080, USA). Tumor Necrosis Factor Alpha ELISA kit was purchased from (Elabscience Biotechnology Company, USA, Catalog Number: E-EL-R2856). Aspartate Amino Transaminase (AST, ALT), ALP, Albumin, Total Protein, and Total Antioxidant Capacity kits for Colorimetric determination of their activities in serum were purchased from bio diagnostic company, Egypt, Catalog Number: AT 10 34 (45), AP 10 20, AB 10 10, TP 2020, and TA 25 13 respectively.

2.6. Histopathological Examination

Small tissue specimens' liver of rats in different groups were collected and immediately fixed in 10% neutral buffered formalin. The tissue specimens were prepared and stained with picrosirius red stain and hematoxylin & eosin stain.

***2.7.** Statistical Analysis*

Data were analyzed using the Statistical Package for the Social Science (S.P.S.S. version 26). Results were expressed as the mean ± SE (standard error). Statistical analysis was done using analysis of one-way variance (ANOVA) followed by Tukey's multiple comparison test to test the difference between groups, the values in each group have characterized by a normal distribution and identical variance. While for the non-parametric data, the equality of groups' means was additionally checked by the Kruskal-Wallis one-way ANOVA by ranks and multiple comparison tests. The level of significance was set at $p<0.05$, $p<0.01$, and $p<0.001$.

3. Results and Discussion

3.1. Results

3.1.1. MEL-loaded chitosan-TPP nanoparticles properties

- *Particle Size Distribution*

The particle size histogram of MEL-loaded Cs-NPs (Figure 1) shows that particle sizes vary from 26 to 49 nm, with an average of 37.5 nm, although very small particles (21–24 nm) have also been detected. Nearly 80% of the particles in the 31 to 44 nm range, according to the frequency distribution seen in the histogram.

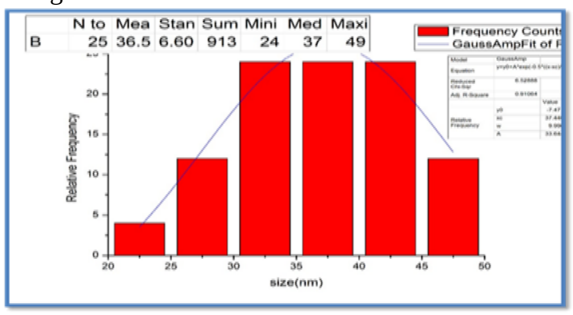

Fig 1. Histogram of Particle size distribution curve of Melittin loaded Chitosan nanoparticles from TEM.

- *TEM morphology*

TEM measurements were used to determine the morphology and shape of nanoparticles. Low magnification (at 200, 500 nm scale) and high magnification (10 nm scale) TEM Nano graphs (Figure 2) revealed that the MEL-loaded Cs-NPs are

spherical in shape, homogenous structure, and uniformly distributed (monodispersed).

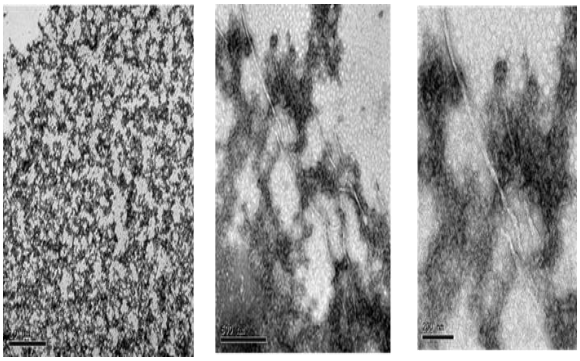

Fig 2. Morphological characterization of MEL loaded Cs-NPs by Transmission electron microscope (TEM), The average diameter of these nanoparticles is 37.5nm and their shape is typically spherical.

• *Fourier-Transform Infrared (FT-IR) Analysis*

FT-IR spectra of Chitosan, MEL, and MEL-loaded CS NPs are presented in (Figure 3). The results showed that the intense characteristic peaks of CS Figure 3(a) were appearing at 3432 cm−1 (−OH), 3356 and 3284 cm−1 (−NH2 stretching), 2967 and 2880 cm−1 (−CH stretching), 1647 cm−1 (C=O carbonyl of the sugar), 1377 and 1254 cm−1 (C–N), and 1070 cm−1 (C−O−C). Figure 3(b) shows that the characteristics peaks of MEL were appearing at 3291 cm−1 (−NH stretching), 2960, 2872 cm−1 (−CH2 aliphatic), 1646, 1536 cm−1 (−C=O stretching), and 1458, 1403 cm−1 (COO−). For MEL-loaded CS NPs Figure 3(c), the intense peak of 3352 cm−1 was become broader, indicating that hydrogen bonding has been enhanced between the −OH bending groups of MEL and CS at 3432 cm−1. The amide I and amide II bending vibrations in CS spectra shifted from 3356 cm−1 and 3284 cm−1 to 3296 cm−1 and 3251 cm−1, respectively, which indicated that some interaction has occurred between −NH3+ groups of CS with TPP and MEL within MEL-loaded CS NPs spectra. The peaks at 2917 cm−1, 2874 cm−1 (−CH aliphatic stretching), 1630 cm−1 (−C=O amidic) and the peaks at 1377, 1254 cm−1 (C–N bending) in CS spectra shifted to 1399, 1253 cm−1 in MEL-loaded CS NPs spectra Figure 3(c). This indicated interaction between the −C=O group of MEL and the primary amide group of CS. The results may indicate that MEL was successfully loaded into CS NPs.

The treatment with MEL Nano (T3) reduced the mean value of ALP level more than the treatment

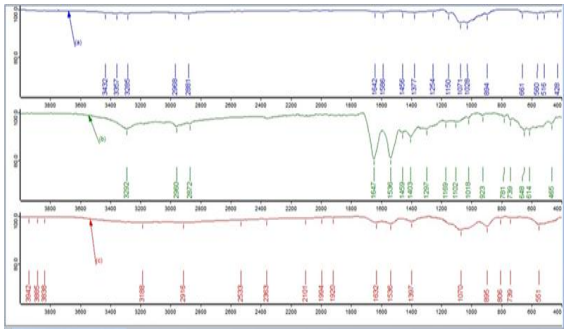

Fig 3. FT-IR spectra of (a) Chitosan, (b) MEL and (c) MEL loaded Cs-NPs.

• *The encapsulation efficiency of MEL-loaded chitosan-TPP nanoparticles*

This nano-formulation was centrifuged and collected after the fabrication of MEL-loaded chitosan-TPP nanoparticles. A spectrophotometer was used to determine the amount of MEL remaining in the supernatant of the solution. The encapsulation efficiency (EE%) for MEL was determined at 82.35%.

3.1.2. Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded CS NPs (40 µg/kg b.w) on liver enzymes

- *ALT level*

Table (1) indicates that the ALT level (U/mL) in the serum has a highly significant increase (***P < 0.001) in the mean value when compared control group to treated1(ZEA only). On the other hand, the treatment with MEL Nano (T3) caused a highly significant decrease (###P < 0.001) in the mean value of ALT level relative to the treatment with MEL only (T2) compared to the (T1) group.

- *AST level*

Table (1) represent the AST level (U/mL) in the serum. There was a highly significant increase (***P < 0.001) in the mean value when compared control group to treated1(ZEA only), While The treatment with MEL Nano (T3), has a highly significant decrease (###P < 0.001) in the mean value more than treatment with MEL only (T2) comparing to (T1) group.

- *ALP level*

Table (1) showed that the ALP activity (IU/L) was highly significantly increased (***P < 0.001) in the mean value after administration of ZEA (T1). While with MEL only (T2) compared to the (T1) group (###P < 0.001).

Table 1: Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded CS NPs (40 µg/kg b.w) on liver enzymes of female rats.

Parameters/Groups	Control	T1 ZEA Only	T2 ZEA+MEL	T3 ZEA+ MEL Nano
ALT(U/mL)	0.58 ± 0.04	2.59 ± 0.12***	0.95 ± 0.04 ###	0.49 ± 0.03 ###
AST(U/mL)	0.66 ± 0.059	2.57 ± 0.11***	0.96 ± 0.08 ###	0.50 ± 0.07 ###
ALP(IU/L)	78.44 ± 1.66	161.60 ± 3.83***	96.08 ± 2.09 ###	72.58 ± 2.57 ###

Values are represented by mean of 12 Rat ± S.E.; Ns= P > 0.05 (Non Significant), *= P ≤ 0.05 (Significant), **= P ≤ 0.01 (Very Significant), ***= P ≤ 0.001 (Highly Significant). *When compared to normal control group, #When compared to ZEA group.

3.1.3. Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded CS NPs (40 µg/kg b.w) on liver synthetic function (production of plasma proteins)

- *Albumin & Total Protein Concentration*

The activities of the liver synthetic function biomarkers Albumin and the total protein in rats treated and control groups are presented in Table (2) and figure (4). The oral administration of ZEA (T1) caused a highly significant decrease in Albumin and Total protein concentration (***P < 0.001) compared to the control group, but the oral administration of MEL Nano (T3) caused a significant increase in the concentration of Albumin (###P > 0.001) and protein in serum more than oral administration of MEL only (T2) compared to the (T1) group after one month of treatment. However, the treatment with MEL Nano (T3) significantly reduced the adverse effects of ZEA at the end of treatment more than treatment with MEL only (T2).

Table 2: Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded CS NPs (40 µg/kg b.w) on liver synthetic function (production of plasma proteins) of female rats.

Parameters/Groups	Control	T1 ZEA Only	T2 ZEA+MEL	T3 ZEA+ MEL Nano
Albumin(g/dL)	**3.56 ± 0.11**	**2.02 ± 0.10*** **	**3.97 ± 0.08###**	**3.99 ± 0.06 ###**
Total Protein(g/dL)	**3.50 ± 0.075**	**1.71 ± 0.086*** **	**3.14 ± 0.13 ###**	**3.44 ± 0.09###**

Values are represented by mean of 12 Rat ± S.E.; Ns= P > 0.05 (Non Significant), *= P ≤ 0.05 (Significant), **= P ≤ 0.01 (Very Significant), ***= P ≤ 0.001 (Highly Significant). *When compared to normal control group, #When compared to ZEA group.

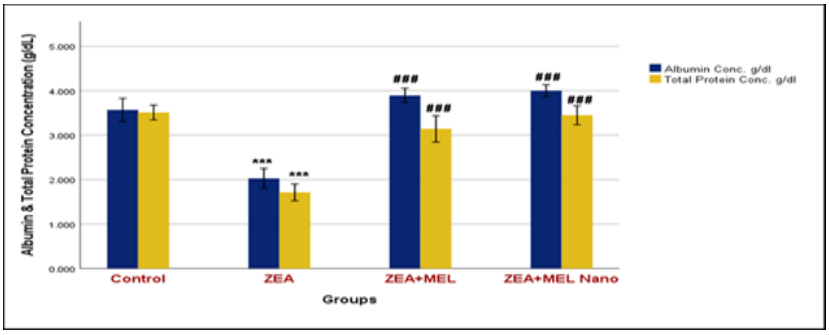

Fig 4. Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL -loaded CS-NPs (40 µg/kg b.w) on Albumin and Total protein in studied groups. Error bars indicate the standard error of the mean.

3.1.4. Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded-CS-NPs (40 µg/kg b.w) on some serum parameters of female rats

- *AFP concentration*

Table (3) showed that the AFP concentration (ng/ml) was highly significantly increased (***P < 0.001) after administration of ZEA for two weeks (T1) compared with the control group, While The treatment with MEL Nano (T3) show a high significant decrease (###P < 0.001) in the mean value of AFP
concentration comparing to (T1) group. but the treatment with MEL only (T2) did not show a significant difference (P < 0.05) in the mean value of AFP concentration comparable to that treated only by ZEA (T1).

- *TNF-α concentration*

Table (3) indicates that the TNF-α concentration (pg/ml) in the serum has a highly significant increase (***P < 0.001) in the mean value when compared control group to (T1) group. While The treatment with MEL Nano (T3) shows a highly significant decrease in the mean value of TNF-α concentration more than the treatment with MEL only (T2) compared to the (T1) group (###P < 0.001).

- *Total-anti oxidant concentration*

Analysis of antioxidant concentration (mM/L) in the serum of both treated and control groups was presented in Table (3) which revealed that the total-anti oxidant concentration has a high significant decrease (***P < 0.001) in the mean value of ZEA (T1) group compared to control. A highly significant increase (###P < 0.001) was observed in the mean value of the MEL Nano (T3) treatment group more than treatment with the MEL only (T2) group compared to the group that was treated only with ZEA (T1).

Table 3: Effect of ZEA (2.7 mg/kg b.w.), MEL (40 µg/kg b.w) and MEL-loaded CS NPs (40 µg/kg b.w) on some serum parameters of female rats

Parameters /Groups	**Control**	**T1 ZEA Only**	**T2 ZEA+MEL**	**T3 ZEA+ MEL Nano**
AFP(ng/ml)	30.63±2.4	57.14±5.46***	56.04±11.56**NS**[a]	29.68±3.24###
TNF-α(pg/ml)	68.20±20.4	286.64±53.9***	57.01±5.17###	40.70±6.29###
Total-anti oxidant(mM/L)	1.91±0.015	1.45±0.025 ***	1.78±0.05###	1.90±.0180###

Values are represented by mean of 12 Rat ± S.E.; *= P ≤ 0.05 (Significant), **= P ≤ 0.01 (Very Significant), ***= P ≤ 0.001 (Highly Significant). *When compared to normal control group, #When compared to ZEA group. (NSa P > 0.05 (Non-Significant) vs. ZEA).

3.1.5. Histopathological Examination

A histological study in the present work was done to give evidence for biochemical results.

I. Assessment of histological changes using H&E stain:

Examination of paraffin sections of control and treated groups of rat's liver stained with hematoxylin & eosin and photographed at different powers; Lowx100 and High x400 at both central vein (CV) and portal area (PA) regions (Figure 5) which showed that compared to normal hepatic tissue structure and normal appearance of hepatocytes with their acidophilic cytoplasm and vesicular active nuclei. T1: Rats were given orally 2.7 mg/kg b.w. of ZEA, two doses (Twice a week) for two weeks; (Chronic dose), showed alteration of normal structure in the form of vascular congestion of both central and portal area vessels, increased chromatin granules within most nuclei. Hepatocytes necrosis with loss of outlines and nuclei. Hepatic sinusoids showed dark acidophilic degenerated cells. Administration of MEL (T2) or MEL-loaded Cs-NPs (T3) as a treatment following ZEA administration results in a marked amelioration of degenerative changes with preservation of the normal hepatic structure. The

superior response was observed in the latter group (T3).

Control group: Showed regular arrangement of hepatocytes with their lightly stained acidophilic cytoplasm and vesicular active nuclei (Thin Black arrows) around CV and PA. The cell plates are separated by thin blood sinusoids (white arrows).

T1 group (ZEA): showed marked vascular congestion of both CV and pA (Black arrows). Hepatocyte nuclear changes in the form of increased chromatin condensation, hepatocytes necrosis (dotted arrows), and the appearance of dark red degenerated cells in hepatic blood sinusoids (thick black arrows).

T2 group (ZEA+MEL); therapeutic group: Marked preservation was observed from degenerative changes induced by ZEA except of residual scattered few hepatocytes necrosis and presence of the dark degenerated cells in blood sinusoids (thick black arrows).

T3 group (ZEA+MEL Nano); Nano therapeutic group: showed significant restoration of the normal structure of hepatocytes both in CV and PA regions, where cells looked even more healthy and viable than control.

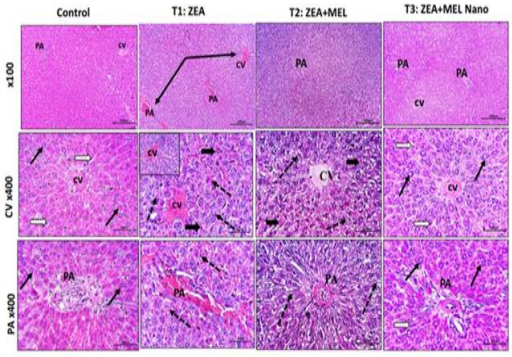

Fig 5. Representative histopathological pictures of liver stained by H & E for control and treated groups. Magnification; Low x100 and High x400 at both central vein (CV) and portal area (PA) regions.

II. Assessments of liver collagen fibers distribution using picrosirius red stain:

Examination of paraffin sections of control and treated groups of rat's liver stained with picrosirius red for collagen (stained red) and photographed at x400 magnification at both central vein (CV) and portal area (PA) regions ***(Figure 6)*** which showed that there is no massive fibrosis of liver parenchyma. A moderate increase of collagen could be detected by a picrosirius red stain around the central vein (CV) and portal area (PA) contents. Administration of MEL only Slightly decreased collagen fibers in the previous regions while administration of MEL nano Was found to markedly decrease collagen fiber deposition to a nearly normal appearance.

Control group: Showed very scanty red stain collagen fibers around CV and PA.

T1 group (ZEA): showed an increase in red-stained collagen deposited around both CV and PA regions.

T2 group (ZEA+MEL); therapeutic group: showed a moderate decrease in collagen fibers around CV and PA regions.

T3 group (ZEA+MEL Nano); Nano therapeutic group: showed evident decrease in collagen fibers around CV and PA regions.

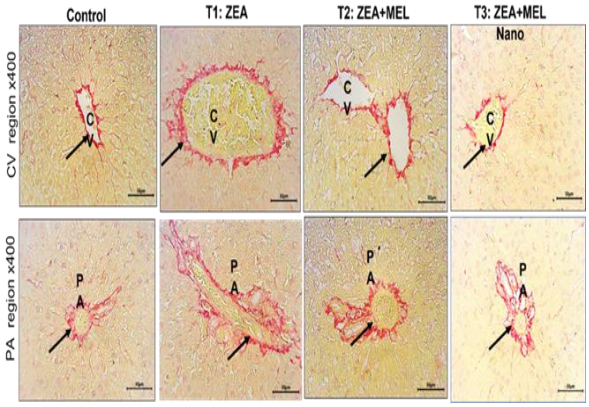

Fig 6. Representative histopathological Sections of rat liver stained by picrosirius red stain for collagen (stained red) for control and treated groups at x400 magnification at both central vein (CV) and portal area (PA) regions.

3.2. Discussion

ZEA is one of the mycotoxins that are from corn, sorghum, and wheat. It has the ability to cause liver lesions and modify some enzymatic parameters of hepatic function [29]. the current research has focused on the hepatotoxic induced by ZEA, and also focused on ZEA toxicity treatment by using free MEL and MEL-loaded-Cs NPs in the liver of female rats. MEL is a major component in the venom of the honeybee Apis mellifera [11]. It has been suggested that melittin has more than one effect, inclusive of anti-bacterial, anti-viral, anti-tumor agent, and anti-inflammatory-responses in various cell types [30]. Furthermore, immuno-conjugation, nanotechnology, and gene therapy are being used to develop MEL-based therapies with increased specificity, selectivity, reduced toxicity, and limited off-target cytolysis [31]. our research is the first report to assess the

ameliorative effect of MEL-loaded-Cs NPs against hepatotoxicity induced by ZEA.

The ionic gelation process was used to load MEL into chitosan-TPP nanoparticles in this study. For MEL-loaded chitosan-TPP nanoparticles, TEM indicated an average diameter of 37.5 nm and a spherical shape, which indicates the best formulation size, where nanoparticle sizes range from 1 nm to 100 nm [32]. By contrast, Medeiros and Silva., [33] loaded MEL on Cs-NPs with a size of 141.8 nm. Moghaddam et al., [34] also produced MEL in niosomes nanoparticles; Their nanoparticles average diameter was 121.4 nm with a spherical shape, while MEL-loaded Cs-NPs with a size of 37.5 nm were made in our research.

In the present study, the encapsulation efficiency of MEL in chitosan-TPP nanoparticles was determined at 82.35%; while in Moghaddam et al., study the entrapment efficiency was less than 79.32% [34]. Probably, using the TPP cross-linker caused to increase of MEL loading in chitosan-TPP nanoparticles. The peaks characterized with the aid of using FTIR evaluation indicated that chitosan changed into connected to TPP salt by ammonium groups of chitosan and phosphate groups of TPP [35]. Our study has indicated that MEL was more linked to chitosan via the hydroxide groups of MEL and ammonium groups of chitosan, so our results show that MEL was successfully loaded into CS NPs.

For the mentioned results, we used free MEL and MEL-loaded Cs-NPs to detoxification of ZEA as mycotoxin from the liver of female rats. We observed that detoxification of ZEA by MEL-loaded Cs-NPs was significantly better than individual melittin. Nanoparticles in drug delivery systems are able to diminish the defects of usual delivery systems [18] and it is probably due to the gradual release of MEL by nanoparticles in infection areas. Accordingly, a combination of chitosan-TPP nanoparticles and MEL produces a synergy effect on the removal of adverse effect of ZEA that is more than free MEL.

The current study demonstrated that when female rats were orally intoxicated with 2.7 mg/kg ZEA for two weeks, we showed a highly significant increase in liver enzymes as AST, ALT, and ALP activities in ZEA (T1) group compared to the control group. We suggested that significantly higher activities of AST, ALT, and ALP in ZEA only (T1) group resulted from the increased permeability of hepatocyte cell membrane which may occur due to the toxic effect of ZEA which cause cellular damage and in turn leakage of the enzymes outside the cellular structure to extracellular fluid. Our results at the end of the experiment reflect hepatocellular damage. Stadnik and Borzecki, [36] supported that increased serum of these enzymes is associated with an increased risk of hepatotoxic which according to this work results. These results were confirmed by Madeha et al., [37] who reported the increase in the activities of AST, ALT, and ALP enzymes causes toxic effects on the liver. In contrast, Treatment with MEL-loaded Cs-NPs has shown highly significantly reduced the serum levels of ALT, AST, and ALP more than the treatment with free MEL, evidencing the ameliorative effects of MEL Nano than free MEL. MEL Nano has been shown to prevent the destruction of liver enzymes in the serum and thus protect the hepatocytes from ZEA-induced hepatotoxicity. These findings were in similarity to the results of Naji et al., [38] who reported that the treatment with MEL reduced the elevation of AST, ALT, and ALP activities, they suggested that the administration of MEL may contribute to protecting liver epithelial cells from damage through the inhibition of inflammatory cytokines and apoptosis.

In our study, we indicated a highly significant decrease in the concentration of Albumin and total protein in the ZEA (T1) group compared to the control group. The liver produces the majority of serum proteins, and the overall amount of proteins in the blood is a primary indicator of liver function. The amounts of these proteins can also change due to a variety of disorders or tissue injury. Albumin and total protein levels were sampled to assess hepatic protein production [39]. We suggested that a significantly higher decrease of albumin and total protein in ZEA only (T1) group at the end of the experiment was indicative of considerable hypoproteinemia and this further confirmed that the liver cells were damaged due to the loss of proteins through the gastrointestinal tract exceeds the synthesis of proteins by the body induced by ZEA, were in conditions causing inflammation and erosions, the mucosal permeability of the gastrointestinal tract increases, leading to excessive leakage of serum proteins into the gut, and poor reabsorption. Normally most of the proteins entering the gut are degraded into amino acids and are reabsorbed [40]. Our results are consistent with the results of Sun et al., [41] who reported that serum levels of albumin and total protein were demonstrated to be significantly decreased in ZEA treated group when compared with control group and they suggested the decreased levels of albumin and total protein may be the result of reduced protein synthesis. Similar results were observed by Liang et al., [42] who demonstrated that after different single doses of ZEA were injected into mice, the levels of albumin and total protein were decreased. In addition,

male Balb/c mice that received an oral treatment of ZEA had significantly decreased albumin and total protein concentration [43]. In contrast, Free MEL and MEL Nano treatment elevated the reduction of albumin and total protein concentration; with MEL Nano (T3) group showing greater improvement than the free MEL (T2) group. From the results obtained, MEL Nano treatment provided a better elevation in serum levels of the albumin and total protein when compared to free MEL treatment. This indicates that MEL Nano is more efficacious than free MEL for elevation of the biochemical parameters induced by ZEA.

In the current study, the level of AFP was increased after administration of ZEA for two weeks (T1) group. AFP is a specific biomarker for liver cancer that is synthesized mainly in the fetal stage [44]. Normally, no production of this marker occurs in the adult cells, but it can be secreted when these cells are transformed into cancer cells. A similar finding was observed by Madeha et al., [37] and EL-Sawi et al., [20] who found that ZEA feeding to male mice for two weeks produced an elevation in the level of serum AFP. Our result suggested a high level of AFP can be a sign of liver cancer, as well as noncancerous liver diseases such as cirrhosis and hepatitis. In contrast, Treatment with MEL-loaded Cs-NPs has shown highly significantly reduced the serum level of AFP, evidencing the ameliorative effects of MEL Nano. While the AFP level is not affected by the treatment with the free MEL (T2) group.

In this investigation, the level of TNF-α was a highly significant increase in ZEA (T1) group compared to the control group. TNF-α mediates cellular interaction in a variety of immunological events and is required for normal hepatocyte proliferation during liver regeneration [45]. High concentrations of TNF-α triggered hepatic cell death, but low concentrations of TNF-α could promote hepatic cell survival [45]. Our findings were in line with Pistol et al., [46] who found that ZEA increased TNF-α in the spleen of animals fed with ZEA. In contrast, Free MEL/MEL Nano treatment reduced TNF-α concentration; with MEL Nano (T3) group showing greater improvement than the free MEL (T2) group. From the results obtained, MEL Nano treatment provided a better reduction in serum level of TNF-α when compared to free MEL treatment. This indicates that MEL Nano could promote hepatic cell survival more than free MEL,

due to their small size and large surface area, drug nanoparticles show increased solubility and thus enhanced bioavailability, additional ability to cross the blood barrier [47].

In our research, after two weeks of ZEA administration, a decrease in the total antioxidant level was observed in the ZEA-treated rats (T1) group. The drop in antioxidant levels can be interpreted as a sign of ZEA-induced tissue damage. Mycotoxins produce oxidative stress by releasing free radicals, inducing lipid peroxidation, and altering the antioxidant state of cells [48, 49]. Free radicals and other reactive molecules were recognized to be neutralized by antioxidants. They can act at any step in the oxidative process, including removing oxygen or lowering local oxygen concentrations, removing catalytic metal ions, and eliminating important reactive oxygen species (ROS) such as O2 and H2O2. Antioxidants protection can operate at several different levels within cells [50, 51]. The intracellular redox state is characterized by the balance of oxidant production and the antioxidant capacity of the cell based on a variety of antioxidants enzymes such as total antioxidants including superoxide dismutase (which reduce O2 to H2O2), catalase and glutathione peroxidase (which reduces H2O2 to H2O) [52]. In agreement with Damiano et al., [53] who explored a low level of antioxidants in the mycotoxin treated rats. In this study, the treatment with MEL Nano (T3) revealed a highly significant increase of the antioxidant level more than free MEL (T2), Our findings indicated that MEL Nano inhibits the effects of oxidative stress induced by ZEA. Similarly, Rakha et al., [54] reported that bee venom markedly increased the total antioxidant capacity.

These findings were supported by liver histopathological analysis on HE-stained slides, where the alteration of normal structure in the form of vascular congestion of both central and portal area vessels, increased chromatin granules within most nuclei, and hepatocellular necrosis observed in the ZEA group were consistent with the lesions already reported in the livers of pig treated with ZEA [6]. Picrosirius red staining was utilized to assess changes in the distribution of liver collagen fibers. In the ZEA-treated group, there was an increase in collagen deposition surrounding the central vein and portal area. Collagen deposition in liver disease is due, at least in part, to increased collagen synthesis. Those liver cells are responsible for collagen synthesis and the regulation of the synthesis of this protein in the diseased liver [55, 56]. Significant restoration of the normal structure of hepatocytes both in central vein and portal area regions and decrease collagen fiber deposition to a nearly normal appearance was reported in the rats of the ZEA+MEL Nano group, where cells looked even more healthy and viable than the ZEA+MEL group compared to the ZEA group.

4. Conclusion

our results further strengthened the role of MEL-loaded Cs-NPs in reducing chronic liver injury, attenuating hepatic inflammatory responses, and inhibiting hepatocyte apoptosis. Thus, it is clear from our findings that MEL-loaded Cs-NPs might possess an ameliorative role in biochemical and histological alterations against hepatotoxicity induced by ZEA.

Abbreviations

ZEA: Zearalenone, MEL: Melittin, Cs-NPs: Chitosan Nanoparticles, TPP: Tripolyphosphate, UV-Vis: Ultraviolet–visible Spectroscopy, FT-IR: Fourier-Transform Infrared, TEM: Transmission electron microscopy, EE: Encapsulation Efficiency, AFP: Alpha-fetoprotein, ALP: Alkaline Phosphatase, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, TNF-α: Tumor Necrosis Factor alpha, TAC: Total Antioxidant Capacity, H&E stain: Hematoxylin & Eosin stain, CV: Central Vein, PA: Portal Area, ROS: Reactive Oxygen Species.

Declarations

Ethics approval and consent to participate

All procedures for using experimental animals were checked and permitted by the "Ethics and Animal Care Committee of Suez Canal University, Ismailia, Egypt" which is fully accredited by the Committee for Purpose of Control and Supervision on Experimental Animals.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Authors' contributions

NM conceived the study, performed experiments, performed blood biochemical and tumor marker analysis, and drafted and wrote the manuscript. OM participated in the design of the study and in experiments and participated in manuscript writing. AM performed melittin-loaded chitosan-TPP nanoparticles properities analysis, and data analysis and participated in manuscript writing. SS performed the histological examination of the liver and participated in manuscript writing. All authors read and approved the final manuscript.

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