https://doi.org/10.48048/tis.2023.6909

Green Synthesis of Zinc Oxide-Nanoparticles from *Ziziphus Spina-Christi* Leaves Extract: Characterization and Their Protective Effects Against Liver Disturbances in Adenine-Exposed Male Rats

Hayder Hussein Abbas[1,*], Narjis Hadi AL-Saadi[1] and Ayyed Hameed Hassan[2]

[1]*Department of Chemistry, College of Sciences, University of Kerbala, Kerbala 56001, Iraq*
[2]*College of Dentistry, University of Kerbala, Kerbala 56001, Iraq*

([*]Corresponding author's e-mail: hayderh.abbas@uokufa.edu.iq)

Received: 25 March 2023, Revised: 3 May 2023, Accepted: 24 May 2023, Published: 31 July 2023

Abstract

Zinc oxide nanoparticles (ZnO-NPs) have a significant feature due to their uses in various fields including medicine, biosensors, food additives, and electronic materials. This study aimed to the green synthesis of ZnO-NPs using aqueous extract of *Ziziphus-spina christi* leaves (Sider) and evaluates its protective roles against liver disorders after adenine-exposed male rats. Thirty-six male rats (3 months in age and weighing 200 ± 5 g) were randomly divided into 6 equal groups as following: Control adenine, *Ziziphus-spina christi*, ZnO-NPs, adenine + *Ziziphus-spina christi* and adenine + ZnO-NPs for 30 days. At the end of 30 days, the activity of (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD) and concentrations of reduced glutathione (GSH) and nitric oxide (NO) were determined, as well as the liver histopathological alterations were investigated. Moreover, the body weight of all rats was measured over a week. The synthesized ZnO-NPs was characterized using several techniques. UV-Visible analysis showed a specific absorption peak at 362 nm. In Fourier Transform Infrared spectroscopy (FT-IR) absorption bands of Zn-O stretching bonding showed between 400 and 500 cm^{-1}. Their crystallinity, hexagonal shape, size, and the specific surface area for the dry ZnO-NPs were also determined using the techniques of X-ray diffraction (XRD) and scanning electron microscopy (SEM) respectively. Results showed significant decreases in ALT, AST and ALP in both groups adenine + *Ziziphus-spina christi* leaves extract and adenine + ZnO-NPs in comparison to the adenine group ($p < 0.05$), as well as significant increases in body weight. Significant decrease was also demonstrated in NO level associated with an increase in SOD, and GSH. Based on these results, it can be said that ZnO-NPs and *Ziziphus-spina christi* leaves effectively protect against adenine-induced liver disorders, these were confirmed by improvements marked in histological changes of liver tissue of rats.

Keywords: Zinc oxide nanoparticles, *Ziziphus spina-christi*, Adenine, Liver, Histopathological study

Introduction

Nanoparticles are involving a high number of atoms at their surface that result in increased surface of reaction. Nanoparticles have several interests and can enter the body in different ways such as injection, ingestion, and inhalation, and then transport to blood may be led to either positive or negative feedback in several organs [1]. There are previous researchers focus on the biological trace element of the body which are usually found in foods or added as a food supplement. However, the mutual actions of the nanoparticles with the biological systems have different unpredictable results, therefore, knowledge of their benefits from their disadvantages is very important to protect the human body from their dangerous effects. Based on that, in the year 2011, the European Parliament called for additional examinations of sufficient safety assessment of the nanofood and demanded that all nanofood should be labeled [2]. Regrettably, most of the markets intentionally or unintentionally did not follow these new rules, and until now no specific labels to indicate if the food contains nanomaterials.

The activity of some metal oxide nanoparticles like zinc oxide nanoparticles make them widely used in various applications involving therapeutics, diagnostics, and nanomedicine based on anti-inflammatory, antioxidant and antimicrobial agents. In order to enhance the beneficial effects of ZnO-NPs and reduce any negative feedback on the health, ZnO-NPs are often used as delivery of the zinc and is listed by the FDA as "Generally Recognized as Safe (GRAS)". ZnO-NPs with small size, makes zinc more easily to be absorbed by the body. Thus, commonly used as a food additive [3]. Besides, ZnO-NPs

is used to preserve various foods and extend shelf life for a longer period. Both the dried powders and suspensions of ZnO-NPs at a specific concentration have been studied and proved their ability to act as antibacterial agent. Previous studies confirmed that ZnO-NPs are effective for inhibiting the growth of both Gram-positive and Gram-negative bacteria.

In several studies, the extracts of different plants parts (root, stalk, leaves, fruits, and seeds) have been applied for the biosynthesis of ZnO-NPs, such *Abelmoschus esculentus* (okra) *mucilage* [4], *Cuminum cyminum* (cumin) [5], *Calotropis gigantea* leaves [6], *Aloe socotrina* leaf [7], *Nigella sativa* seed [8] and *Ziziphus jujube* (Sider or Nabq) [9]. Plant extracts are rich in several organic compounds, involving flavonoids, alkaloids, phenolic compounds, sterols, saponin, tannins, and fatty acids which act as capping and stabilizing agents [10], which stabilized the synthesized nanoparticles, and prevent the particles from agglomeration. *Ziziphus-spina christi* is an excellent example and a very common plant is grown in several areas of Iraq that are used extensively for its health properties, and the medicinal benefits of *Ziziphus-spina christi* are anti-inflammatory, antioxidant, antimicrobial, and anticancer.

Accordingly, the aim of the current study is the biosynthesis of ZnO-NPs using an aqueous extract of *Ziziphus-spina christi* leaves. The ZnO-NPs synthesized were characterized using various characterization techniques to examine their morphological, physical, and chemical properties, then used to study their protective effect on the liver in adenine-exposed male rats.

Materials and method

Chemical and kits

Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) was obtained from CDH Company (India), adenine with high purity grade was purchased from Solarbio Company (China), dimethyl sulfoxide (DMSO) was obtained from Xi`an Sheerherb Biological Technology Company (China). Kits for ALT, AST, and ALP were supplied by Solarbio Company (China). Kits for superoxide dismutase (SOD) activity assay, reduced glutathione (GSH) content assay, and nitric oxide (NO) content assay were obtained from Solarbio Company (China). Ethanol (96 % v/v) was supplied by Fluka Co. (Switzerland), and sodium hydroxide (NaOH 99 %) was purchased from BDH Company (U.K.).

Preparation of crude extract of *Ziziphus-spina christi* leaves

Fresh leaves of *Ziziphus-spina christi* were collected from the College of Medicine-University of Kufa. The fresh leaves were thoroughly washed under tap water to remove the adhered followed by distilled and de-ionized water to remove dust and other particles. Then, they were air dried and ground into a powder using a mortar and pestle. After that, 10 gm of powdered leaves were soaked in 100 mL de-ionized water in conical flasks. Then the plant material was heating-stirrer at 45 °C for 15 min, then filtered through muslin cloth, and centrifuged at 1,500 rpm for 10 min. The crude extract was dried in the oven for 30 min at 45 °C to make powder for further use.

Preparation and purification of zinc oxide nanoparticles

To prepare of ZnO-NPs, 90 mL of 1 mM aqueous solution of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was prepared in de-ionized water and 10 mL aqueous extract of *Ziziphus-spina christi* leaves. The mixture was adjusted to pH 9 using 0.5M NaOH, and then heated at the temperature of 60 °C with high-speed stirring. The mixture was sealed at this condition for 2 h until the mixture was transformed into a brownish precipitate. The precipitated ZnO-NPs were cleaned with de-ionized water followed by ethanol and then dried in a warm air oven at the temperature of 100 °C for 90 min followed by the calcination step at 300 °C for 1 h. The obtaining yellowish-white powder of ZnO-NPs that ready for characterization and further uses.

Optimization of the ZnO NPs biosynthesis

Optimization of the reaction conditions involving the volume of *Ziziphus-spina christi* leaves extract, the temperature, and the concentration of zinc salts precursor. These were carried out using a UV-visible spectrophotometer.

Effect of the plant extract volume

Biomass quantity plays a very important role in the synthesis of the nanoparticle. The different volumes of *Ziziphus-spina christi* leaves at 5, 10, and 15 mL were used to synthesize ZnO-NPs and they were characterized using a UV-visible spectrophotometer.

Effect of temperature

Temperature plays a very important role in all reactions. Optimization studies with regard to temperature were carried out with temperatures ranging from 40 - 70 °C with a difference of 10 °C for the synthesis of ZnO-NPs. The sample was analyzed with a UV-visible spectrophotometer.

Effect of $ZnNO_3.6\ H_2O$ concentration

The synthesis of nanoparticles is greatly dependent on the substrate concentration. The concentrations of $ZnNO_3.6\ H_2O$ (1 mM, 2 mL and 3 mM) were studied. The optimum concentration for the production of ZnO-NPs was confirmed using a UV-vis spectrophotometer.

Characterization of zinc oxide nanoparticles

Zinc oxide nanoparticles were characterized using different modern techniques as follows:

UV-visible spectroscopy

The optical properties of ZnO-NPs were evaluated using UV-vis spectrophotometer (UV-1650PC, SHIMADZU, Japan). The spectra were set in between the wavelength of 300 - 700 nm range. In spectrophotometer de-ionized water was set as available reference.

Fourier transform infrared FT-IR spectroscopy

FT-IR spectroscopy was used to determine the various functional groups involved in *Ziziphus-spina christi* leaves extract and synthesized ZnO-NPs. These were investigated using (IRPrestige-21, SHIMADZU, Japan) under the spectral range of (4,000 - 400 cm^{-1}). Identification of the functional groups present in the dried extract and synthesized ZnO-NPs was achieved at room temperature using the KBr pellet method. The presence of the various vibrational modes was investigated.

X-ray diffraction (XRD)

To determine the average crystalline size of biosynthesized ZnO-NPs and identification its crystalline features were characterized using XRD technique using (XRD-600, SHIMADZU, Japan) with Cu Kα radiation (Voltage = 40 kV, Current =30 mA, λ=1.5406 Å, scan rate of 5.0 ° min^{-1} and scan range of 2 θ from 20 - 80 °). From the XRD data obtained, the crystalline size of the synthesized ZnO-NPs was calculated according to Debye-Scherrer's equation.

Scanning electron microscopy (SEM)

The surface morphology of green synthesized ZnO-NPs was performed by using scanning electron microscope (TESCAN MIRA3-SEM, Czech Republic). Zinc oxide nanoparticles was added into SEM slides to made thin layer. Then the slide was set for SEM analysis after coating it with carbon copper grids. After that ZnO-NPs was observed under SEM at increasing the voltage of 20 KV, and the images were recorded.

Experimental animals

Thirty-six male rats weighing (200 ± 5 g) and approximately (6 - 7) weeks old used in this study were purchased from the animal house at the College of Sciences-University of Kufa. The experiment was carried out at the Laboratory Animal Facilities, College of Sciences, University of Kufa, the animals were kept under observation for 2 weeks before the beginning of the experiment. The animals were housed in plastic cages 50×35×15 cm^3 and were maintained under controlled conditions of temperature (25 ± 2 °C) with the cycle of light/dark for 12 h [11].

Experimental design

Rats were randomly divided into 6 groups (6 rats each).

1) First group (G-I): Rats were orally given 0.5 mL DMSO (5 % v/v) for 30 days and served as controls.

2) Second group (G-II): Rats were orally given 0.5 mL adenine (100 mg/kg.BW) dissolved in DMSO daily for 30 days to induce liver damage. The dose of adenine was chosen from the previous study by Za'abi *et al.* [12].

3) Third group (G-III): Rats gavaged 0.5 Ml of *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) daily for 30 days [13].

4) Fourth group (G-IV): Rats orally received 0.5 mL of ZnO-NPs (10 mg/kg B.W) daily for 30 days [14].

5) Fifth group (G-V): Rats in this group were co-administrated 0.25 mL adenine (100 mg/kg.BW) and 0.25 mL *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) at the same time for 30 days [15].

6) Sixth group (G-VI): Rats were co-administrated with 0.25 mL adenine (100 mg/kg.BW) and 0.25 mL of ZnO-NPs (10 mg/kg B.W) at the same time for 30 days.

Collection of blood

Blood specimens

At the end of 30 days, rats were anesthetized by placing them in a closed jar containing cotton rinsed with chloroform to be sedated for the next step, which is blood collection via cardiac puncture in sterile syringes by needle prick in the heart draining 2 - 3 mL of blood carefully, then blood placed in a test tube containing gel which leaves for 30 min at room temperature, and then used for obtaining serum by centrifuging at 3,000 for 15 min. The serum was then divided into 1.5 mL Eppendorf tubes and stored at (−20 °C) for further examinations.

Tissue sampling

After blood collection, rats were sacrificed to remove their liver and then fixed in buffered-formalin solution (10 %) at room temperature. After fixation, the liver specimens were dehydrated, cleared, embedded in paraffin wax, and then prepared for histological examination using hematoxylin and eosin stain (H&E), and observed with a light microscope (Olympus BH-2, Tokyo, Japan) for assessment of histological alterations. The histopathological changes were scored in order to perform the comparison between the groups.

Biochemical analysis

Evaluation of liver function parameters

The serums were kept at (−20 °C) and used for the determination of hepatic enzymes ALT, AST and ALP spectrophotometrically by appropriate commercial kits.

Assessments of antioxidants statues

The antioxidants statues were determined, superoxide dismutase (SOD) activity, reduced glutathione (GSH), and nitric oxide (NO) levels were determined spectrophotometrically using a commercial assay kit.

Statistical analysis

Liver function parameters were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey's post hoc test to determine the differences between the averages with values of ($p < 0.05$) were considered statistically significant. The data were expressed as mean ± standard deviation (SD) of studied groups. The statistical analysis was performed using Statistical Package for the Social Sciences Software (SPSS)Version (19, Inc., Chicago, IL, USA).

Results

The results showed successfully synthesis of ZnO-NPs and the first indication for synthesized it was the change of color from light yellow to brownish within 2 h (**Figure 1**). The colorimetric change was detected using a UV-visible spectrophotometer.

Figure 1 The gradual change in the color of the synthesized ZnO NPs from *Ziziphus-spina christi* leaves extract and zinc nitrate hexahydrate with time (0 min - 48 h).

Optimization of biosynthesized ZnO-NPs

Optimization of reaction conditions such as the volume of *Ziziphus-spina christi* leaves extract, temperature, and substrate concentration were carried out using a UV- visible spectrophotometer.

Effect of plant extract quantity

The UV- visible spectra depicted in **Figure 2(a)**, describes the impact of varying volumes of aqueous extract on the biosynthesis of ZnO-NPs. A steady improvement in the absorption peak was observed when increasing the extract volume from 5 to 10 mL. Maximum absorption was observed with 10 mL of *Ziziphus-spina christi* leaves extract in 1 mM of zinc nitrate hexahydrate. Any increase or decrease in this volume led to decrease in the absorption values.

Effect of temperature

Temperature is one of the vital factors affecting the ZnO-NPs formation. The effect of varying temperatures on the synthesis of ZnO-NPs using *Ziziphus-spina christi* leaves extract is carried out at different temperatures from 40 to 70 °C and the maximum production of ZnO-NPs was obtained at 60 °C in 1 mM concentration compared to other temperatures and also sharp peak was detected by UV-vis spectrophotometer at 362 nm. On the other side, it is clear that with the rise in temperature from 60 °C, the absorption bands of ZnO-NPs showed similar patterns with high and extremely broad peaks (**Figure 2(b)**).

Effect of precursor metal ion concentration

Another important parameter in obtaining the optimum biosynthesis is the concentration of zinc nitrate hexahydrate on the synthesis of ZnO-NPs. From the UV-vis spectra, it is clear that, the optimum substrate concentration required was 1 mM using UV-vis spectrophotometer at the maximum wavelength of 362 nm. Further increase in the concentration to 2 and 3 mM led to a shift towards broader wavelength due to the increase in particle size (**Figure 2(c)**).

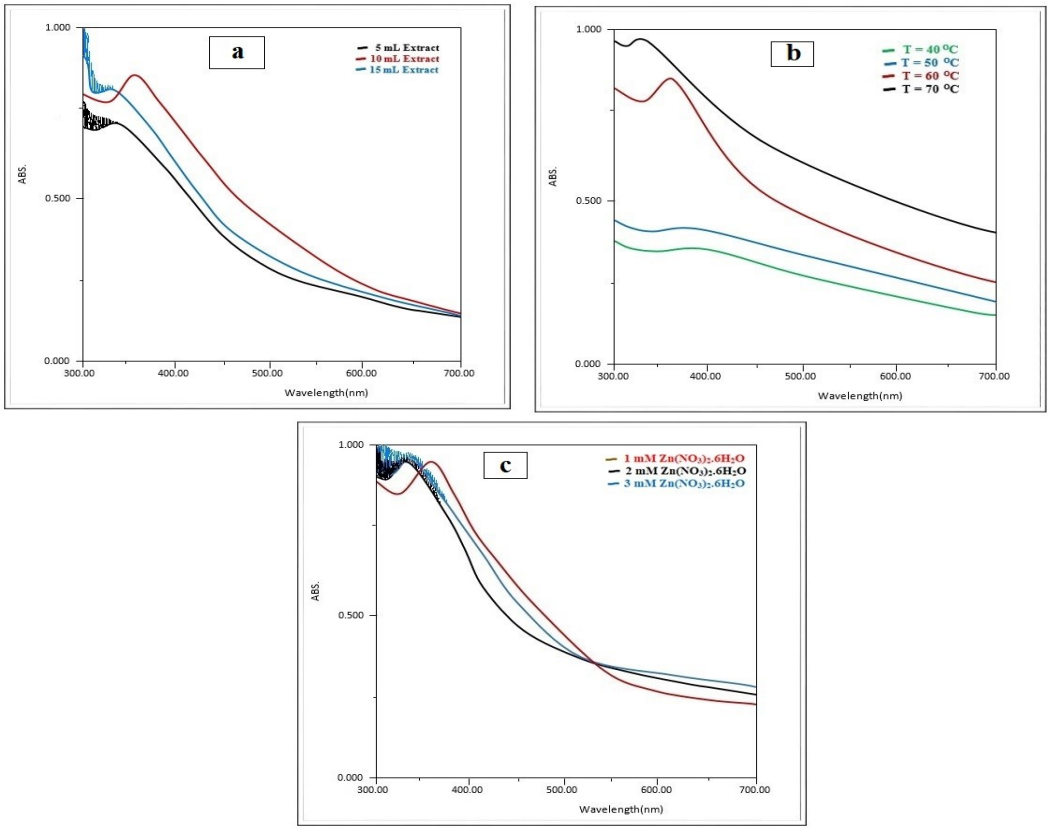

Figure 2 (a) Effect of extract volume, (b) Effect of temperature, and (c) Effect of precursor metal ion concentration.

Characterization of zinc oxide nanoparticles

Ultraviolet-visible spectroscopy (uv-vis spectroscopy)

In this study, the optimal formation of ZnO-NPs was obtained by the color change was confirmed using the UV-Vis spectrophotometer in range of 200 - 700 nm. Absorption peaks showed in the UV-Vis spectrum (**Figure 3**) represents the synthesized ZnO-NPs at 362 nm after 48 h incubation at room temperature.

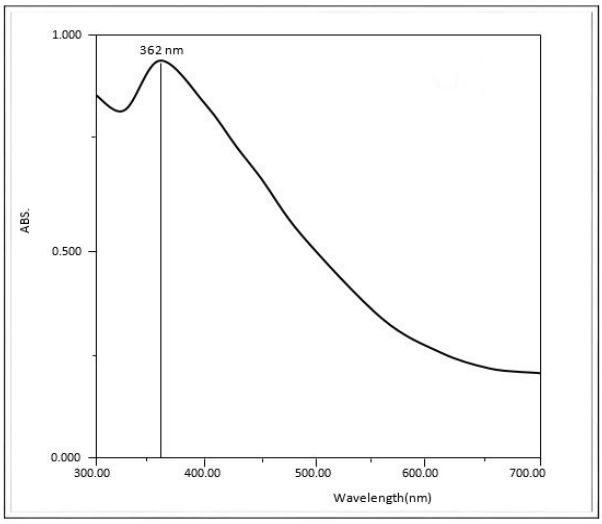

Figure 3 UV-visible spectra of the synthesized ZnO-NPs at 362 nm.

Fourier transform infrared spectroscopy (FT-IR)

Figure 4 represent the FT-IR spectra of *Ziziphus-spina christi* leaves extract, as well as the synthesized ZnO-NPs. Spectrum of *Ziziphus-spina christi* leaves extract (**Figure 4(a)**) showed a strong absorption peak at 3,377.36 cm^{-1} was resulted from stretching of the O-H groups due to the presence of alcohols, phenolic compounds and carbohydrates [16]. While the peak at 2,917.94 and 1,648.58 cm^{-1} related to the stretching vibration of (= C–H) and (C = O), and the peak at 1,588.63 cm^{-1} also be related to the surface adsorbed water molecule. The peak between 2,917.94 and 2,612.71 cm^{-1} were correspond to the symmetric and asymmetric of stretching vibrations of -C-H in $-CH_3$ and $-CH_2-$ of aliphatic hydrocarbons chains. Similarly, the bands at 1,572.48 cm^{-1} (O–H bending vibrations), 1,388.75 cm^{-1} (C–O stretching of the ester group), 1,255.66 cm^{-1} (C–O asymmetric stretching in cyclic polyphenolic compounds) and 1,058.92 to 619.18 cm^{-1} indicating the presence of C–N stretch (aromatic amines) and alkyl halides respectively. Meanwhile, the FT-IR spectra of the synthesized ZnO-NPs (**Figure 4(b)**) confirmed the phase transformation and formation of ZnO-NPs. Also, showed a wide band at 3,415.83 cm^{-1} that may be related to O-H groups due to the presence of trace amounts of water in ZnO-NPs samples. Absorption bands centered at 1,612.48 and 1,396.46 cm^{-1} which can be assigned to asymmetric and symmetric C = O stretching modes, respectively, due to traces of the *Ziziphus-spina christi* leaves extract, especially with the biosynthesized ZnO-NPs. There are peaks were showed in the region between 451.34 and 424.34 cm^{-1} are specified to vibrational stretching of metal oxygen (Zn-O) that indicated of reducing of Zn^{2+} to Zn and formation of ZnO nanoparticles.

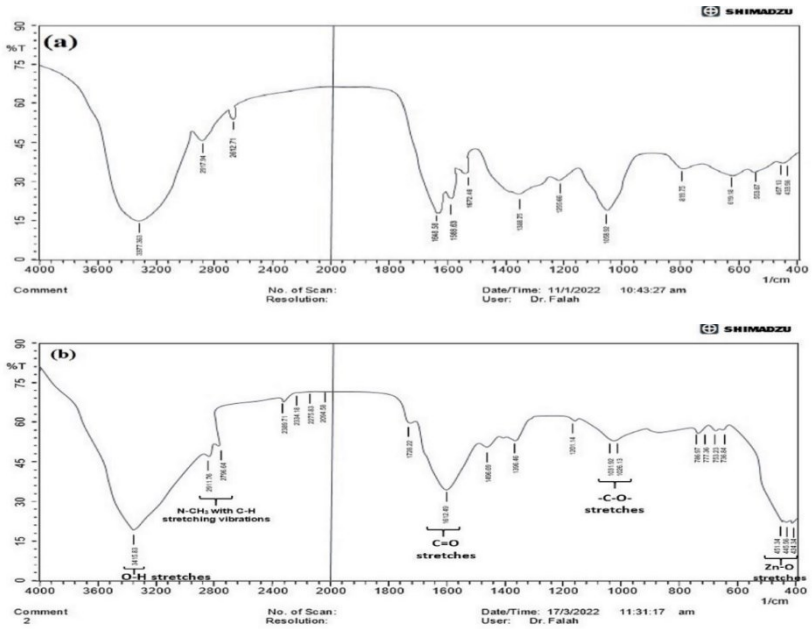

Figure 4 FT-IR spectrum; (a) *Ziziphus-spina christi* leaves extract powder and (b) ZnO-NPs

X-ray diffraction (XRD)

As shown in **Figure 5(a)** the XRD patterns of the prepared ZnO-NPs show characteristic peaks at 2θ angles equals to 31.77, 33.21 and 36.59 ° corresponding to (100), (002) and (101) planes, respectively, relative to hexagonal wurtzite structure when compared with the standard data file of (JCPDS file no. 36-1451) as shown in **Figure 5(b)**. Average crystallite size of ZnO-NPs was calculated applying the Debye-Scherrer's equation [17].

$$D = \frac{k\lambda}{\beta \, Cos\, \theta}$$

where D, k, λ, and β represent the average crystal size, shape factor (0.9), wavelength (0.15416) and Bragg angle θ of the X-ray (1.5406 Å) Cu Ka radiation, respectively. The ZnO-NPs of typical size had been calculated as 38.177 nm, confirming the nano size of the ZnO-NPs.

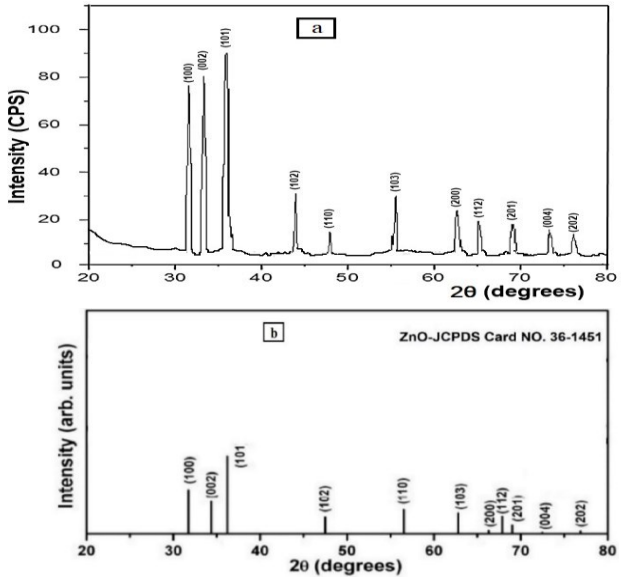

Figure 5 (a) X-Ray diffraction (XRD) of synthesized ZnO-NPs from *Ziziphus-spina christi* leaves extract and (b) Standard card of ZnO-JCPDS Card No. 36-1451.

Scanning electron microscope (SEM)

The surface morphology of the synthesized ZnO-NPs was studied by observing the images captured under SEM using different magnifications, and the results are presented in **Figure 6**. SEM images of ZnO-NPs showed semispherical shape and these particles are in a highly agglomerated form as shown in **Figures 6(a) - 6(b)**. The average particle size was ranged at diameter: D_1 (39.74 nm), D_2 (42.25 nm), and D_3 (48.19 nm) as shown in **Figure 6(c)**. The average particle size was also obtained from the given SEM images with a diameter of 43.39 nm.

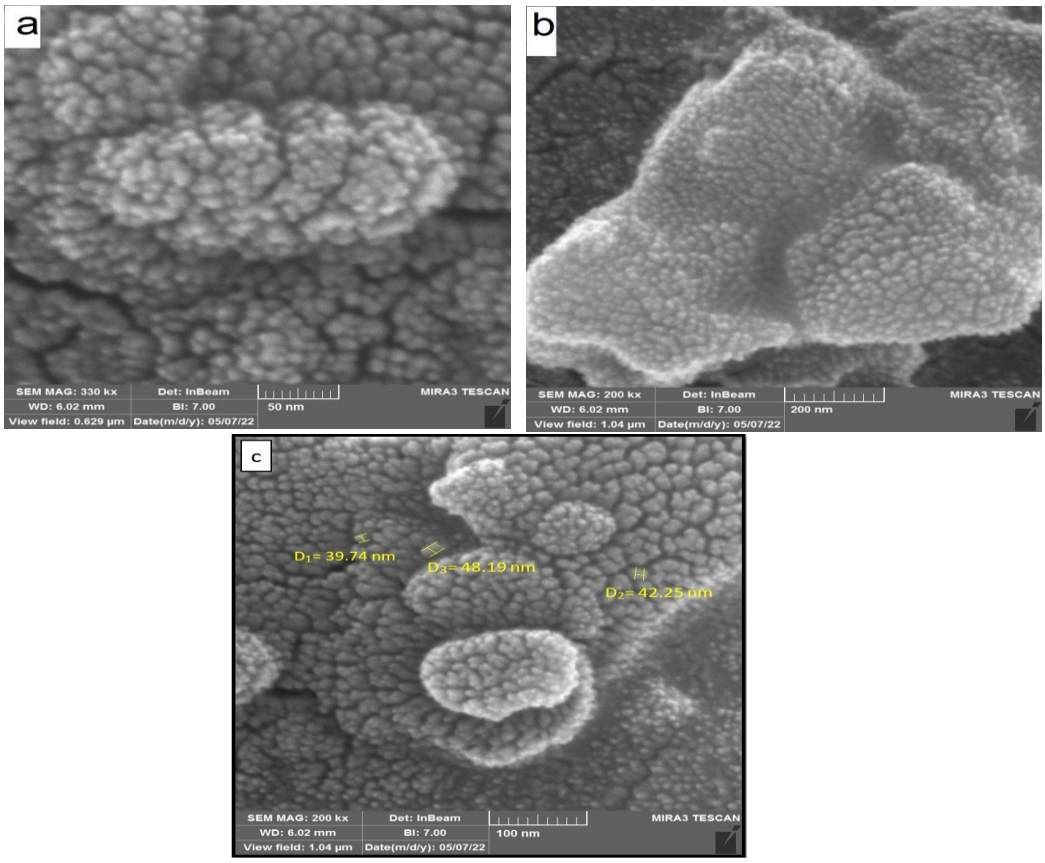

Figure 6 SEM images of synthesized ZnO-NPs at different magnifications (a) at 50 nm, (b) at 200 nm, and (c) SEM images at 100 nm with the particle size at different average diameters D1, D2, and D3.

Effects of ZnO-NPs and *Ziziphus-spina christi* leaves extract on liver biochemical parameters induced by adenine

The present study was designed to evaluate the effects of ZnO-NPs and *Ziziphus-spina christi* leaves extract on hepatic damage induced by adenine in male rats. Adenine induced several pathological markers of liver damage in rats, as shown in the previously published studies [18-20].

Effect on body weight changes

As shown in **Table 1**, rats of the control group were grown by about (10 ± 2 gm) during the 30 days of giving DMSO only. Whereas, the second group rats (G-II) that gavaged adenine (100 mg/kg.BW) for the same period showed a significant decrease ($p < 0.05$) in body weight about (20 ± 2 gm) in comparison to the control group. The same **Table 1** showed the administration of *Ziziphus-spina christi* leaves extract for third group rats (G-III) at a dose (10 mg/kg B.W) significantly slightly decreased ($p < 0.05$) their body weights when compared to the control group, but these weights were high in comparison to adenine group. The same behavior for the body weight of 4 group rats (G-IV) after administrated of ZnO-NPs (10 mg/kg B.W), which is high compared to the adenine group, but ZnO-NPs will significantly decrease ($p < 0.05$) the body weight when compared to a control group. Finally, the 5 and 6 group rats (G-V and G-VI) after co-administration of adenine plus *Ziziphus-spina christi* leaves extract or adenine plus ZnO-NPs at a dose (10 mg/kg B.W) respectively, mitigated significantly the decreasing ($p < 0.05$) in body weights of rats by compared to adenine group rats, but they still lower when compared with control group rats.

Table 1 Effect of ZnO-NPs and *Ziziphus-spina christi* leaves extract on adenine-induced body weight changes.

Period time Groups	Day-0 Weight(gm)	Week-1 Weight(gm)	Week-2 Weight(gm)	Week-3 Weight(gm)	Week-4 Weight(gm)
G-I	204.19 ± 0.32	206.98 ± 0.35	209.74 ± 1.29	212.16 ± 0.96	215.85 ± 0.48
G-II	204.23 ± 0.05	197.52 ± 0.06[a]	193.03 ± 0.26[a]	187.54 ± 0.73[a]	182.38 ± 1.13[a]
G-III	204.27 ± 0.06	205.14 ± 0.46[ab]	207.65 ± 0.57[ab]	209.85 ± 0.59[ab]	211.50 ± 0.67[ab]
G-1V	204.08 ± 0.29	206.13 ± 0.57[ab]	208.96 ± 0.62[ab]	211.35 ± 0.66[ab]	214.69 ± 0.81[ab]
G-V	204.25 ± 0.10	201.16 ± 0.16[ab]	197.23 ± 1.06[ab]	194.41 ± 1.19[ab]	191.26 ± 1.64[ab]
G-VI	204.36 ± 0.43	203.26 ± 0.64[ab]	199.51 ± 0.91[ab]	196.84 ± 0.38[ab]	194.35 ± 0.59[ab]
Tukey test	0.317	0.422	0.846	0.863	1.513

Notes: Values in the table are expressed as mean ± SD. n = 6 male rats in each group, means in the same column with different superscript letter(s) are significantly different, [a]$p < 0.05$ versus the control group. [b]$p < 0.05$ versus the adenine alone. SD: Standard deviation.
G-I: Control group, G-II: Adenine group, G-III: *Ziziphus-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Ziziphus-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

Effect on liver functions enzymes

The results of ALT, AST, and ALP were statistically significant increases ($p < 0.05$) in their activities in the adenine group (G-II) compared with the control group (**Table 2**). Result of the third group rats (G-III) that administrated of *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) showed a significant decreased ($p < 0.05$) in enzymes activities of ALT, AST, and ALP in comparison to the adenine group but were slightly significant differences in comparison to the control group, while the 4 group rats (G-IV) that administrated of ZnO-NPs (10 mg/kg B.W) showed decreasing significantly ($p < 0.05$) in their enzymes activities comparing to adenine group but they were still high as compared to the control group. Furthermore, in the both fifth and 6 group rats (G-V and G-IV) were co-administration of adenine plus *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) or adenine plus ZnO-NPs (10 mg/kg B.W) causes a considerable decrease ($p < 0.05$) in activities of ALT, AST, and ALP when compared with adenine group, but still higher when compared to the control group.

Table 2 Effect of *Ziziphus-spina christi* leaves extract and ZnO-NPs on liver function parameters in adenine-exposed male rats.

Parameters groups	ALT (U/mL)	AST (U/mL)	ALP (U/mL)
G-I	33.94 ± 1.15	103.28 ± 2.17	122.36 ± 2.64
G-II	91.60 ± 3.57[a]	179.46 ± 3.89[a]	213.55 ± 4.18[a]
G-III	36.35 ± 1.23[ab]	108.03 ± 2.36[ab]	125.14 ± 2.79[ab]
G-1V	44.71 ± 1.26[ab]	117.67 ± 2.51[ab]	132.80 ± 2.85[ab]
G-V	60.44 ± 2.28[ab]	144.53 ± 2.92[ab]	183.49 ± 3.92[ab]
G-VI	72.11 ± 2.63[ab]	158.80 ± 3.27[ab]	197.03 ± 4.05[ab]
Tukey test	2.735	3.189	4.096

Notes: Values in the table are expressed as mean+SD. n = 6 male rats in each group, means in the same column with different superscript letter(s) are significantly different, [a]$p < 0.05$ versus the control group. [b]$p < 0.05$ versus the adenine alone. SD: Standard deviation.
ALT = Alanine aminotransaminase, AST = Aspartate aminotransaminase, ALP = alkaline phosphatase. G-I: Control group, G-II: Adenine group, G-III: *Ziziphus-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Ziziphus-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

Effect on antioxidants levels

Table 3 shows the results of antioxidant status, they are SOD activity, GSH, and NO concentrations in the serum of male rats. As shown in the **Table 3**, where noticed that SOD and GSH were remarkably decreased, while NO was significantly increased after administrated of adenine (100 mg/kg.Bw) to second group rats (G-II) in comparison to the control group ($p < 0.05$) . On other hand, administration of *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) for third group rats (G-III) and ZnO-NPs (10 mg/kg B.W) for 4 group rats (G-IV) significantly increased SOD and GSH when compared to the adenine group, but were significantly decreased in comparison with the control group, while the NO was significantly decreased when compared to the adenine group, but still higher in comparison with control group rats ($p < 0.05$). Furthermore, rats of the 5 and 6 groups (G-V and G-VI) after co-administration of adenine plus *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) and adenine plus ZnO-NPs (10 mg/kg B.W) respectively, lead to a significant increase in SOD and GSH compared to adenine group, but still lower in comparison with the control group. This increase was accompanied with a significant decrease in NO when compared to the adenine group but was significantly increased when compared to the control group ($p < 0.05$).

Table 3 The effect of *Ziziphus-spina christi* leaves extract and ZnO-NPs on the antioxidant levels in adenine-exposed male rats.

Parameters groups	SOD (U/mL)	GSH (µmol/L)	NO (µmol/L)
G-I	17.15 ± 0.44	13.48 ± 0.38	0.93 ± 0.01
G-II	8.92 ± 0.14[a]	5.40 ± 0.05[a]	8.54 ± 0.21[a]
G-III	15.23 ± 0.41[ab]	11.35 ± 0.29[ab]	1.47 ± 0.02[ab]
G-1V	13.08 ± 0.33[ab]	9.74 ± 0.26[ab]	1.95 ± 0.02[ab]
G-V	10.73 ± 0.28[ab]	7.55 ± 0.10[ab]	4.52 ± 0.04[ab]
G-VI	9.51 ± 0.24[ab]	6.19 ± 0.08[ab]	6.31 ± 0.10[ab]
Tukey test	0.479	0.355	0.294

Notes: Values in the table are expressed as mean+SD. n = 6 male rats in each group, means in the same column with different superscript letter(s) are significantly different, [a]$p < 0.05$ versus the control group. [b]$P < 0.05$ versus the adenine alone. SD: Standard deviation.

SOD = superoxide dismutase, GSH = reduced glutathione, NO: Nitric oxide. G-I: Control group, G-II: Adenine group, G-III: *Ziziphus-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Ziziphus-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

Histological study

Results of the histological study of liver tissue for all groups of rats after 30 days of exposure were performed using 2 dyes called hematoxylin and eosin (H&E staining), which make easy to see different parts of the tissues under the light microscope (**Figure 7**). The control group rats (G-I) showed the normal central vein, normal arrangement of hepatic cell and normal sinusoids, without any vacuolated of the cytoplasm (**Figure 7(L1)**). The histopathological section in the liver of adenine group rats (G-II) showed a clear alteration including cytoplasm vacuolization, necrosis of hepatocytes, and amyloid like substance precipitation in the wall of liver sinusoids (**Figure 7(L2)**). Third group rats (G-III) were administered of *Ziziphus-spina christi* leaves extract (10 mg/kg B.W) showed normal architectures of the liver, and normal hepatocytes with some areas around the periportal region appear to be mildly dilated (**Figure 7(L3)**). Liver tissue of 4 group rats (G-IV) administrated with ZnO-NPs (10 mg/kg B.W) exhibited similar liver cytoarchitecture compared with the third group rats but the portal areas show mild to moderate round cell inflammatory infiltrate (**Figure 7(L4)**). Finally, in the both fifth and 6 groups rats (G-V and G-VI) were co-administered adenine plus aqueous extract of *Ziziphus-spina christi* leaves (10 mg/kg B.W) or adenine plus ZnO-NPs (10 mg/kg B.W) showed reduced congestion of the hepatic central vein, the cytoplasm not vacuolated. Sinusoids are well protected with reduced inflammation hepatocytes (**Figures 7(L5) - 7(L6)**).

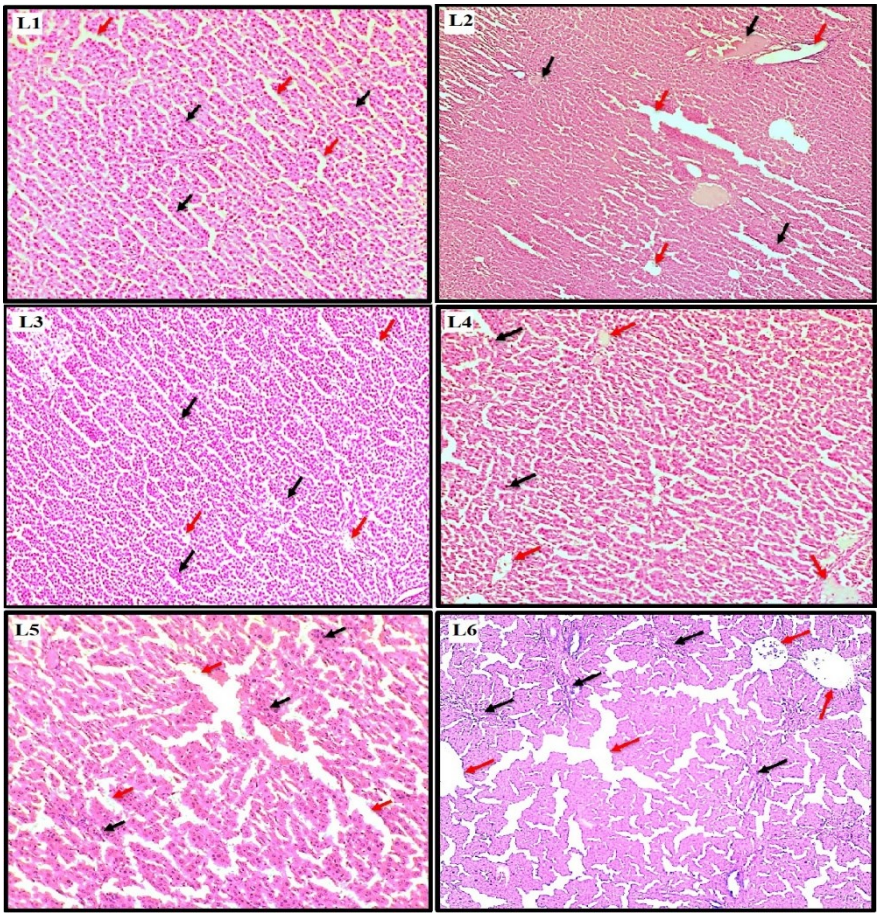

Figure 7 Effects of ZnO-NPs and *Ziziphus-spina christi* leaves extract on the histological appearance of liver tissues in adenine- exposed male rats. The tissue is stained by H&E satin and the section is captured using light microscope and digital camera at 10X magnification scale. L1: Liver of control group rats were gavaged with DMSO only showed the normal hepatocytes (black arrows) and normal central vein (Red arrows), without any significant occupied lesion (SOL). L2: Liver of adenine group rats showed clear damage in the hepatocytes (Black arrows) and clear thrombi formation can be seen in the hepatic central vein in section (Red arrows). L3: Liver of male rats were administrated with aqueous extract of *Ziziphus-spina christi* leaves extract showed a normal hepatocyte (Black arrows) and normal radial arrangement around central vein (Red arrows). L4: Liver of male rats were administrated with ZnO-NPs showed slightly thrombi of hepatocytes in some areas (Black arrows) and central vein (Red arrows). L5: Liver of male rats were treated with (Adenine + *Ziziphus-spina christi* leaves extract) showed infiltration and necrosis in the hepatocytes (Black arrows) and the clear damage of central vein in some areas. L6: Liver of male rats were treated with (Adenine + ZnO-NPs) shows infiltration in hepatic tissue and coagulative necrosis of the hepatocytes (Black arrows) and clear damage in the wall of central vein (Red arrows).

Discussion

Zinc oxide nanoparticles were successfully biosynthesized using the plant extracts. Plant extracts include the various phytochemicals such as flavonoids, alkaloids, phenolic compounds, tannins, amino acids, and carbohydrates, etc. which play as effective reducing as well as capping agent without addition of any further chemical components [21]. Most of these contents have biological activity. *Ziziphus-spina christi* leaves have several applications and have a range of beneficial biological activities including anti-inflammatory, antioxidant, and anti-arthritic activities, and used in the preparation of pharmaceutical drugs [22]. The major phytochemical component of *Ziziphus-spina christi* leaves are flavonoids, tannins, and phenolic compounds which may act as bio-reducing and stabilizing agents, due to the availability of OH groups, for nanoparticle synthesis [23].

The first evidence for the green synthesis of ZnO nanoparticles is the change in color into brownish due to the reducing of Zn^{2+} to Zn. The crude extract of *Ziziphus-spina christi* is considered as reducing agent for Zn^{2+} and a chemical stabilizer for ZnO-NPs. Synthesis of ZnO-NPs is based on how long the reducing and stabilizer agents are exposed to metal ions. Therefore, when the incubation period of zinc with the reducing agent increases, the synthesis of ZnO-NPs would further increase [24].

Characterization of ZnO-NPs is essential to assure its synthesis and know whether the preparation is suitable for a specific application. UV-vis spectroscopy exhibited a strong absorption peak at a wavelength of 362 nm was attributable to present ZnO-NPs (**Figure 2**).

To estimate of the best conditions for ZnO-NPs, various effectives factors were studied, temperature, filtrate volumes for leaves extract, and concentrations of zinc nitrate hexahydrate. The optimal formation of ZnO-NPs was obtained by using 10 mL aqueous extract of *Ziziphus-spina christi* leaves, a 1 mM of zinc nitrate hexahydrate and the temperature of reaction mixture at 60 °C, all these conditions were the best and selected for synthesizing ZnO-NPs. These conditions were chosen due to the rapid changing in color of the reaction mixture and increased absorbance which indicated increases in ZnO-NPs formation.

Fourier transform infrared (FT-IR) analysis was performed to identify the functional groups and determine the interaction of nanoparticles with phytochemicals. These biomolecules act as capping, reducing and stabilizer agents to formation metal-nanoparticles [25]. FT-IR measurement of *Ziziphus-spina christi* leaves extract (**Figure 3(a)**) showed several peaks that represent different functional groups such as O-H group at 3,377.36 cm^{-1} and C = O group at 1,648.58 cm^{-1}. These active groups were shifted to lower frequency at 3,415.83 and 1,612.48 cm^{-1} respectively, indicates the participation of these compounds in the synthesis of ZnO-NPs and are responsible for the capping, performance, stabilization and prevent agglomeration of nanoparticles. The bending and stretching vibration show that biomolecules such as phenolic groups, alcohol, carboxylic acid, and proteins are responsible in the reduction Zn^{2+} and stabilizing of the ZnO-NPs. The peaks at 451.34, 445.56 and 424.34 cm^{-1} (**Figure 3(b)**), which resulting from the vibrational stretching of covalent bond between zinc metal and oxygen atom (Zn-O) that strongly confirmed the formation of ZnO-NPs. Locations and intensities of absorption peaks in the FT-IR spectrum of *Ziziphus-spina christi* leaves extract were compared to those in the FT-IR spectrum of ZnO-NPs synthesized, results showed that some of the peak's position and absorption intensities in the plant extract spectrum were replicated in ZnO-NPs spectrum. As showed from these findings, it can be concluded that the ZnO-NPs synthesized are non-oxidative, pure, and coated with contents of *Ziziphus-spina christi* leaves extract.

X-Ray diffraction analysis of ZnO-NPs synthesized from *Ziziphus-spina christi* leaves extract, where X-ray deviation configurations of ZnO-NPs detect that peaks were in agreement with the standard data card of (JCPDS Card No. 36-1451) (**Figure 4(b)**). The presence of strong intensity and narrow width of diffraction peaks of 31.77 ° (100), 33.21 ° (002), and 36.59 ° (101) planes in XRD patterns indicates the formation of high purity and crystalline of the ZnO-NPs, these peaks were wonderfully arranged with the hexagonal crystal structure [26]. The average crystallite size was estimated using Debye-Scherrer's equation formula, and it was found to be 38.177 nm. Hence, we can conclude that *Ziziphus-spina christi* is primarily responsible for the reduction of Zn^{+2} and production of ZnO-NPs. The increase in crystallite size of ZnO-NPs might be attributed to the existence of the long chain natural products such as the polyphenols in *Ziziphus-spina christi* leaves extract which act as capping agents and prevent the agglomeration of the ZnO-NPs and due to the thermal treatment via the microwave heating at high temperatures [27].

SEM imagines of ZnO-NPs (**Figure 6**) showed the high density spherical shaped nanoparticles. A huge number of ZnO-NPs were aggregated forming a nano-form like morphology with various irregular shapes of agglomerated particles. Aggregation could be due to a high surface energy of ZnO-NPs and also may be due to intensification as the result of narrow space between nanoparticles. These strongly confirm the particles were presented in a homogeneous form and this homogeneity plays as an important role in the different activities of ZnO-NPs.

The study effect of ZnO-NPs and *Ziziphus-spina christi* leaves extract on the liver in adenine-exposed male rats. As known that adenine leads to hepatic malfunction because of an increase in the pro-inflammatory cytokines and free radicals by the formation of uremic toxins. Adenine-exposed male rats showed markers of hepatic malfunction, which suffered from high serum ALT, AST, and ALP [28], where these enzymes are more credible signs of hepatocyte harm or necrosis. Their levels are raised in various hepatic diseases. Of the 3 enzymes, ALT is considered the most specific for hepatic injury due to its presence mainly in liver cytosol and in low concentration somewhere else.

The body weight of rats was measured for 4 weeks, and it was showed that increases the growth of rats in the control group (G-I) fed with DMSO and normal diet, linearly increased from the first day to the last day of the experiments. Adenine group rats (G-II) exhibit a significant decrease in body weight as compared to the control group rats ($p < 0.05$), this may be attributed to the toxicity generated by the exposure to adenine resulted in the production of reactive oxygen species (ROS), inflammation and metabolic disorder caused oxidative damage to the cells and organs and disturbance of the immune system [29]. Slightly significant retardation in growth was found in the plant extract group (G-III) and ZnO-NPs Group (G-IV) in comparison to the control group ($p < 0.05$). Both treatment groups rats, the adenine plus plant extract group (G-V) and adenine plus ZnO-NPs group (G-VI) showed improvement in growth and increase body weight when compared to the adenine group ($p < 0.05$). These changes in body weight may be due to reducing the accumulation of free radicals and oxidative stress.

The measurement of enzyme activities ALT, AST, and ALP play a significant role in diagnosis, disease investigation, and the assessment of the plant extracts or nanoparticles for safety and toxicity risk. These enzymes considered in this study are useful marker of liver cytolysis and damage. ALT is found in many organs, particularly in the liver for diagnostic use, when its elevation indicates hepatocyte damage and release into the plasma. AST is not a specific enzyme for the liver only but is also founded in several organs such as the kidney, heart, brain, and skeletal muscle, these organs when are destroyed, AST is released. ALP is the indication enzyme for the plasma membrane and is elevated in a variety of tissues. It is often used to estimate the safety of plasma membranes and as a marker for hepatobiliary diseases [30]. Tissue damage is usually associated with the release of enzymes from the affected organ or tissue into circulation. Exposure of rats to adenine led to cytotoxicity in a time and dose dependent as a consequence of the oxidative stress, the peroxidation of lipids, and the damage of carbohydrates, proteins, and cell membranes. Adenine caused a significant increase in serum levels of ALT, AST, and ALP (**Table 2**). Increases in serum ALT and AST activities have been indicated in conditions involving necrosis of hepatocytes, while the increases of serum ALP activity has been implicated in hepatobiliary diseases. The decrease ($p < 0.05$) in activities of ALT, AST and ALP in the liver of rats were administrated with *Ziziphus-spina christi* leaves extract or ZnO-NPs (**Table 2**) when compared to the adenine group might be according to the inactivation of these enzymes by the extract or its metabolites, which could have suppressed the synthesis of the enzymes, also suggested that ZnO-NPs might be interaction with key molecules in membranes (enzymes inclusive) and then inhibited or denature them. Furthermore, in both the treated groups (5 and 6) after co-administration of adenine plus *Ziziphus-spina christi* leaves extract or adenine plus ZnO-NPs showed a significant decrease ($p < 0.05$) in serum ALT, AST, and ALP activities indicating the strong evidence of the protective effect by the plant extract and ZnO-NPs. The significant decrease in ALT, AST, and ALP enzymes activities in the fifth group rats were treated with *Ziziphus-spina christi* leaves extract at a dose (10 mg/kg B.W) and 6 group rats were treated with ZnO-NPs at dose (10 mg/kg B.W), these when comparison with adenine group rats, these results were agreements with [31]. The alteration in the activities of the enzymes in this study might be due to the biological activity of ZnO-NPs and phytochemicals (main components of *Ziziphus-spina christi* leaves) in the doses given for 30 days are capable to decreasing serum ALT, AST and ALP.

As shown in **Table 3**, rats were exposed to adenine, SOD and GSH was significantly decreased in serum, this is associated with the significant increase of NO content. Therefore, an increase in of NO level considered as a marker of oxidative damage that reveal excess of free radical production, which is consistent with the view that adenine induces lipid peroxidation. The failure of antioxidant defense mechanism is due to the decreased activities of the scavenging enzymes, or both. Increased NO levels, lipid peroxidation, also decreased SOD and GSH levels are associated with cell damage. These results supported [32-33] who reported that adenine significantly increased oxidative stress markers and decreased the activities of antioxidant enzymes in serum.

Rats were treated with an aqueous extract of *Ziziphus-spina christi* leaves was ameliorated the change in antioxidant statutes. This amelioration appeared by the increase in serum levels of SOD, and GSH is associated with decreasing NO levels, which further confirms their role against adenine-induced liver damage. Administration of ZnO-NPs showed approximately a similar activity (**Table 3**), to that of aqueous extract of *Ziziphus-spina christi* leaves in adenine-treated rats. Finally, ZnO-NPs protected rats against oxidative damage and liver injury induced by adenine which may be due to the anti-inflammatory and antioxidant properties that protect cell membranes against oxidative damage, decrease free radicals and nitric oxide (NO) levels, and increase the antioxidant enzyme as superoxide dismutase (SOD) levels [34,35]. Moreover, as shown in **Table 3**, *Ziziphus-spina christi* leaves extract and ZnO-NPs were able to significantly decrease liver damage induced by adenine, but observed that co-administration of adenine plus *Ziziphus-spina christi* leaves extract (G-V) gave better results when compared to the adenine plus

ZnO-NPs (G-VI). Also, improvement in antioxidant statutes might be attributed to the anti-inflammatory and antioxidant properties of phytochemicals which protect against the loss of functional safety of the hepatocytes.

The histological changes of the liver were harmonic with those results observed in the various biochemical parameters, the results were supported by increases in the liver enzyme activities as mentioned in **Table 2**. The histological section of the liver demonstrates adenine-induced periportal inflammation, sinusoidal congestion hemorrhage, and hepatic necrosis in the liver of rats. These results were in agreement with [36]. Liver damage was possible due to the direct toxic effects of adenine-mediated oxidative stress on the hepatic cell. Group rats were co-administered with adenine plus aqueous extract of *Ziziphus-spina christi* leaves appear the significant improvement compared with adenine-treated rats as indicated by the contrary periportal decreased inflammatory hepatic cells, decreased vacuolization and sinusoidal congestion in liver cells. Co-administration of adenine plus ZnO-NPs were enhanced the hepatic necrosis, central vein, and sinusoidal congestion in comparison to adenine-treated rats. Moreover, administration of *Ziziphus-spina christi* leaves extract alone or ZnO-NPs alone displayed approximately similar results as that showed in the control group. These results were in agreement with previous study [15].

Conclusions

The current study indicates that the synthesized ZnO-NPs and aqueous leaf extract of *Ziziphus spina-christi* at (10 mg/kg B.W) respectively exhibit hepatoprotective effects against adenine-induced liver damage, implying that they may be used safely against liver disorders at this concentration; no significant effects were observed in normal hepatocytes, implying that they may be a powerful antioxidant, anti-inflammatory, and antitoxic agents. Thus, these nanoparticles should be recorded for its global acceptance as dependent drug.

Acknowledgements

The authors would like to acknowledge the Management of the central laboratory- Faculty of Pharmacy and laboratory of animal facilities-College of Sciences-University of Kufa, Kufa, Iraq for introducing the assistance and providing necessary facilities for research work. We also wish to special thanks to Dr. Hussam Raheem Mohammed, College of Medicine - University of Missan, Missan, Iraq for his valuable discussions and support.

References

[1] VD Matteis. Exposure to inorganic nanoparticles: Routes of entry, immune response, biodistribution and *in vitro*/*in vivo* toxicity evaluation. *Toxics* 2017; **5**, 29.

[2] Y Wanga, L Yuana, C Yaoa, L Dinga, C Lia, J Fanga, K Suia,Y Liub and M Wua. A combined toxicity study of zinc oxide nanoparticles and vitamin C in food additives. *Nanoscale* 2014; **6**, 15333-42.

[3] MA Pourmirzaiee, S Chehrazi, M Heidari-Beni and R Kelishadi. Serum zinc level and eating behaviors in children receiving zinc supplements without physician prescription. *Adv. Biomed. Res.* 2018; **7**, 120.

[4] AR Prasad, J Garvasis, SK Oruvil and A Joseph. Bio-inspired green synthesis of zinc oxide nanoparticles using Abelmoschus esculentus mucilage and selective degradation of cationic dye pollutants. *J. Phys. Chem. Solid.* 2019; **127**, 265-74.

[5] E Zare, S Pourseyedi, M Khatami and E Darezereshki. Simple biosynthesis of zinc oxide nanoparticles using nature's source, and its *in vitro* bio-activity. *J. Mol. Struct.* 2017; **1146**, 96-103.

[6] SK Chaudhuri and L Malodia. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage. *Appl. Nanoscience* 2017; **7**, 501-12.

[7] BA Fahimmunisha, R Ishwarya, MS AlSalhi, S Devanesan, M Govindarajan and B Vaseeharan. Green fabrication, characterization and antibacterial potential of zinc oxide nanoparticles using Aloe socotrina leaf extract: A novel drug delivery approach. *J. Drug Deliv. Sci. Tech.* 2020; **55**, 101465.

[8] NA Al-Shabib, FM Husain, F Ahmed, RA Khan, I Ahmad, E Alsharaeh, MS Khan, A Hussain, MT Rehman, M Yusuf, I Hassan, JM Khan, GM Ashraf, A Alsalme, MF Al-Ajmi, VV Tarasov and G Aliev. Biogenic synthesis of zinc oxide nanostructures from nigella sativa seed: Prospective role as

food packaging material inhibiting broad-spectrum quorum sensing and biofilm. *Sci Rep.* 2016; **6**, 36761.
[9] M Golmohammadi, M Honarmand and S Ghanbari. A green approach to synthesis of ZnO nanoparticles using jujube fruit extract and their application in photocatalytic degradation of organic dyes. *Spectrochim. Acta Mol. Biomol. Spectros.* 2020; **229**, 117961.
[10] P Basnet, TI Chanu, D Samanta and S Chatterjee. A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents. *J. Photochem. Photobiol. B Biol*. 2018; **183**, 201-21.
[11] E Edition. *Guide for the care and use of laboratory animals*. The National Academies Press. Washington DC, 2021.
[12] MA Za'abi, MA Busaidi, J Yasin, N Schupp, A Nemmar and BH Ali. Development of a new model for the induction of chronic kidney disease via intraperitoneal adenine administration, and the effect of treatment with gum acacia thereon. *Am. J. Translational Res.* 2015; **7**, 28-38.
[13] SAE Bashandy, OAH Ahmed-Farid, S Abdelmottaleb-Moussa, EA Omara, GAA Jaleel and FAA Ibrahim. Efficacy of zinc oxide nanoparticles on hepatocellular carcinomainduced biochemical and trace element alterations in rats. *J. Appl. Pharmaceut. Sci.* 2021; **11**, 108-17.
[14] A Awadalla, ET Hamam, FF El-Senduny, NM Omar, MR Mahdi, N Barakat, OA Ammar, AM Hussein, AA Shokeir and SM Khirallah. Zinc oxide nanoparticles and spironolactone-enhanced Nrf2/HO-1 pathway and inhibited Wnt/β-catenin pathway in adenine-induced nephrotoxicity in rats. *Redox Report* 2022; **27**, 249-58.
[15] SAE Bashandy, A Alaamer, SAA Moussa and EA Omara. Role of zinc oxide nanoparticles in alleviating hepatic fibrosis and nephrotoxicity induced by thioacetamide in rats. *Can. J. Physiol. Pharmacol.* 2018; **96**, 337-44.
[16] X Ji, F Zhang, R Zhang, F Liu, Q Peng and M Wang. An acidic polysaccharide from *Ziziphus Jujuba* cv. Muzao: Purification and structural characterization. *Food Chem.* 2019; **274**, 494-9.
[17] BD Cullity. *Elements of x-ray diffraction (Addison-Wesley series in metallurgy and materials)*. Addison-Wesley Publishing Company, Inc. Boston, 1959.
[18] EA Saad, HA El-Gayar, RS EL-Demerdash and KH Radwan. Hepato-toxic risk of gum arabic during adenine-induced renal toxicity prevention. *J. Appl. Pharmaceut. Sci.* 2018; **8**, 104-11.
[19] AJ Nawfal, MI Younus and AT Yassen. Protective effect of tomato against oxidative damage of liver induced by adenine in male rats. *HIV Nursing* 2022; **22**, 3300-5.
[20] EA Saad, HA El-Gayar, RS El-Demerdash and KH Radwan. Frankincense administration antagonizes adenine-induced chronic renal failure in rats. *Phcog. Mag.* 2018; **14**, 634-40.
[21] MD Abdulrahman, AM Zakariya, HA Hama, SW Hamad, SS Al-Rawi, SW Bradosty and AH Ibrahim. Ethnopharmacology, biological evaluation, and chemical composition of *Ziziphus spina - christi* (L.) Desf.: A review. *Adv. Pharmacol. Pharmaceut. Sci.* 2022; **2022**, 4495688.
[22] SM Khaleel, SJ Jaran and TM Al-Deeb. Antimicrobial and lipid peroxidation inhibition potential of *Ziziphus spina-christi* (sedr), a Jordanian medicinal plant. *J. Biol. Sci.* 2019; **19**, 131-6.
[23] AAA Aljabali, Y Akkam, MSA Zoubi, KM Al-Batayneh, B Al-Trad, OA Alrob, AM Alkilany, M Benamara and DJ Evans. Synthesis of gold nanoparticles using leaf extract of *Ziziphus* and their antimicrobial activity. *Nanomaterials* 2018; **8**, 174.
[24] G Rocchetti, L Lucini, G Chiodelli, G Giuberti, D Montesano, F Masoero and M Trevisan. Impact of boiling on free and bound phenolic profile and antioxidant activity of commercial gluten-free pasta. *Food Res. Int.* 2017; **100**, 69-77.
[25] D Hu, W Si, W Qin, J Jiao, X Li, X Gu and Y Hao. Cucurbita pepo leaf extract induced synthesis of zinc oxide nanoparticles, characterization for the treatment of femoral fracture. *J. Photochem. Photobiol. B Biol.* 2019; **195**, 12-6.
[26] RC Fierascu, A Ortan, SM Avramescu and I Fierascu. Phyto-nanocatalysts: Green synthesis, characterization, and applications. *Molecules* 2019; **24**, 3418.
[27] S Damiano, M Forino, A De, LA Vitali, G Lupidi and O Taglialatela-Scafati. Antioxidant and antibiofilm activities of secondary metabolites from *Ziziphus jujuba* leaves used for infusion preparation. *Food Chem.* 2017; **230**, 24-9.
[28] AC Boon, AK Lam, V Gopalan, IF Benzie, D Briskey, JS Coombes, RG Fassett and AC Bulmer. Endogenously elevated bilirubin modulates kidney function and protects from circulating oxidative stress in a rat model of adenine-induced kidney failure. *Sci Rep.* 2015; **5**, 15482.
[29] D Claramunt, H Gil-Peña, R Fuente, E García-López, V Loredo, O Hernández-Frías, FA Ordoñez, J Rodríguez-Suárez and F Santos. Chronic kidney disease induced by adenine: A suitable model of growth retardation in uremia. *Am. J. Physiol. Ren. Physiol.* 2015; **309**, F57-F62.

[30] P Jarsiah, A Nosrati, A Alizadeh and SMB Hashemi-Soteh. Hepatotoxicity and ALT/AST enzymes activities change in therapeutic and toxic doses consumption of acetaminophen in rats. *Int. Biol. Biomed. J.* 2017; **3**, 119-24.

[31] AB Țigu, AI Moldovan, CS Moldovan, S Pojar, R Drula, CT Jula1, D Gulei, ML Nistor, BP Moldovan1, CȘ. Mirescu and CL Roșioru. Lycopene and phycocyanin-biological properties in experimental diabetes: 2. Effects on biochemical, enzymatic and histological parameters. *Studia Univ. Babeş Bolyai Biol.* 2016; **61**, 41-5.

[32] BH Ali, S Al-Salam, YA Suleimani, JA Kalbani, SA Bahlani, M Ashique, P Manoj, BA Dhahli, NA Abri, HT Naser, J Yasin, A Nemmar and MA Za'abi. Christina hartmann7 and nicole schupp curcumin ameliorates kidney function and oxidative stress in experimental chronic kidney disease. *Basic. Clin Pharmacol. Toxicol.* 2018; **122**, 65-73.

[33] C Xue-ying, L Cui, W Xing-zhi, L Zhang, D Zhu, Z Xiao-rong and H Li-rong. Quercetin attenuates vascular calcification through suppressed oxidative stress in adenine-induced chronic renal failure rats. *BioMed Res. Int.* 2017; **2017**, 5716204.

[34] ZK El-Maddawy and WSHAE Naby. Protective effects of zinc oxide nanoparticles against doxorubicin induced testicular toxicity and DNA damage in male rats. *Toxicol. Res.* 2019; **8**, 654-62.

[35] AA Khalaf, EI Hassanen, RA Azouz, AR Zaki, MA Ibrahim, KY Farroh and MK Galal. Ameliorative effect of zinc oxide nanoparticles against dermal toxicity induced by lead oxide in rats. *Int J. Nanomedicine* 2019; **14**, 7729-41.

[36] MA Za'abi, A Shalaby, P Manoj and BH Ali. The effects of adenine-induced chronic kidney disease on some renal and hepatic function and CYP450 metabolizing enzymes. *Physiol. Res.* 2017; **66**, 263-71.