Nanoparticles for active combination radio mitigating agents of zinc coumarate and zinc caffeinate in a rat model

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Abstract
Zinc coumarate and zinc caffeinate nanoparticles (ZnCoNPs, ZnCaNPs) affect different biological processes. This study aimed to evaluate the mitigating action of ZnCoNPs in combination with ZnCaNPs against liver damage induced by gamma rays (γ-rays). Rats were exposed to 7 Gy of γ-rays and then injected intraperitoneally (i.p) with ZnCoNPs [2U/rat/day (5 mg/kg)] and ZnCaNPs [2U/rat/day (15 mg/kg)] for 7 consecutive days. The results showed that irradiated rats treated with ZnCoNPs (5 mg/kg/body weight) in combination with ZnCaNPs (15 mg/kg/body weight) for 7 days had a significant increases in body weight, antioxidant levels, T helper cell 4 (cluster of differentiation 4 (CD4)), and T cytotoxic cell 8 (cluster of differentiation 8 (CD8)), associated with a marked decrease in lipid peroxidation (LP), nitric oxide(NOx), total free radicals concentrate (TFRC), and DNA fragmentation. There were positive alterations in the morphological state, hematological parameters and the cell cycle phases. Additionally, the histopathological study demonstrated an improvement in the liver tissue of irradiated rats after treatment. Thus, ZnCoNPs and ZnCaNPs could be used as natural mitigating agents to reduce the hazards of ionizing radiation.

Keywords Radiomitigators · Nanoparticles · Antioxidants · DNA damage · Rats

Introduction

Ionizing radiation (IR) is considered a physically powerful agent that causes heritable changes in cells. Nevertheless, it is well applied in diagnosing and treating
human diseases (Elgazzar and Kazem 2006). IR may lead to cancer, mortality, and neural dysfunction in humans and animals. It can also separate the lasting attraction between atoms, ions, or molecules and ionization crucial macromolecules, like nucleic acids, membrane lipids, and proteins (Lavelle and Foray 2014), resulting in reactive oxygen species (ROS), that participate in normal cell damage and also induce DNA damage (Gao et al. 2018).

Radioprotectors and radiomitigators are substances confirmed to alleviate the damage induced by radiation exposure (Citrin et al. 2010). Plants and their extracts are used as phytotherapy against various pathologies (Ferreira et al. 2014). Phenolic compounds like p-coumaric acids (P-CAs) and caffeic acids (CAs) are involved in different pathophysiological mechanisms (Zeb 2020).

P-CAs and CAs are carbon-hydrogen bond compounds that are hydroxyl derivatives of cinnamic acid, with antioxidant, anti-inflammatory, and anticarcinogenic effects (An et al. 2010; Pragasam et al. 2013; Kilani-Jaziriet al. 2017; Benbettaieb et al. 2018; Kheiry et al. 2019; Sabitha et al. 2019; Agunloye et al. 2019; Choi et al. 2019; Kianmehr et al. 2020). Furthermore, previous studies reported their protective properties in the hepatic and renal cells against oxidative injury (Ekinci Akdemir et al. 2017; Tsai et al. 2017). Nanotechnology is the treatment and production of materials on the size of atoms and has possible applications in a wide range of sectors, from energy, electronic materials, optics, and remediation, to food, cosmetics, and medicine (Jeevanandam et al. 2018). In nanoform the natural radiomitigators achieved better stability, lower toxicity, and better penetration and distribution into the tissue (Krokosz et al. 2016).

Thus, the current study planned to assess the mitigating role of a natural radiomitigator compound and zinc coumarate (ZnCoNPs) combined with zinc caffeinate (ZnCaNPs) nanoparticles against liver damage induced by γ-rays in rats. This purpose has been achieved by the determination of antioxidant and oxidant markers, DNA damage, T helper (CD4) and T cytotoxic (CD8), blood parameters, and cell cycle phases. The histopathological study liver tissues confirmed the results.

Material and methods

Chemicals

The acids of p-coumarate and caffeinate were purchased from Sigma, Chemicals (Saint Louis, MO, USA).

Gamma rays

Whole body gamma rays (γ-rays) to rats were made at the National Center for Radiation Research and Technology (NCRRT, Cairo, Egypt), using Canadian 137Cs (half-life time 30.719 years) Gamma cell 40 biological irradiator with energy and activity of 662 keV and 46,625 GBq (equal to 1260 Ci), respectively. The rat’s whole body was irradiated with a single dose of 7 Gy and the dose rate was 0.006 Gy/s.

Animals

Male adult Wistar albino rats weighing 150 ± 5 g were gained from the animal house belonging to the (NCRRT). Animals were maintained among typical circumstances and with standard food and water and a controlled illumination state (light: dark, 13 h: 11 h).

Ethics approval

The animals’ treating procedure has been accepted by the animal ethics committee of the NCRRT per the 3Rs’ principles for animal experimentation prepared by Central Scientific Publishing Committee, Egyptian Atomic Energy Authority (Ref. (190)—7/07/2020).

Rats were injected intraperitoneally with ZnCoNPs [2U/rat/day (5 mg/kg)] and ZnCaNPs [2U/rat/day (15 mg/kg)] (Ekinci Akdemir et al. 2017; Pari and Prasath 2008).

Experimental design

Rats were separated into four groups (eight rats each) as follows:

Group 1 (control): normal healthy rats
Group 2 (ZnCoNPs + ZnCaNPs): rats were injected with zinc coumarate nanoparticles (5 mg/kg/body weight) combined with zinc caffeinate nanoparticles (15 mg/kg/body weight)
Group 3 (IR): the whole body of rats was irradiated with a single dose of 7 Gy
Group 4 (IR+ZnCoNPs+ZnCaNPs): rats were irradiated with 7 Gy of γ-rays and then were injected with zinc coumarate nanoparticles (5 mg/kg/body weight) combined with zinc caffeinate nanoparticles (15 mg/kg/body weight) for 7 consecutive days.

Sample preparation

Animals were under urethane anesthetic (1.2 g/kg, intraperitoneally). Consequently, blood was collected using a
sterilized syringe from the heart puncture after 7 days of irradiation and a fasting period of 12 h. The first part of the blood will be collected in heparin and ethylenediaminetetraacetic acid (EDTA) tubes. The second part was centrifuged at 4000 \( \times g \) for 15 min to obtain serum for the biochemical study. The animals were then scarified; the liver was cut, washed in saline, and weighed; and 10% (w/v) tissue homogenates were made in 0.1-M phosphate buffer (pH 7.4) by Teflon Homogenizer (Glas-Col, Terre Haute, IN, USA). The supernatant result was centrifuged at 10,000 \( \times g \) for the biochemical study.

**Preparation of ZnCoNPs**

NaOH (1.2 g) was dissolved in distilled water, and 5 g of \( p \)-coumaric acid was added. Moreover, the reaction mixture was mixed by stirring over a hot plate to complete sodium coumarate formation. ZnCl\(_2\) (4.1 g) was dissolved in distilled water, and followed by sodium coumarate was added to obtain yellow precipitate from zinc coumarate separated by filtering, rinsing, and drying in the oven. Moreover, zinc coumarate powder was crushed in the mill to convert into nanoscale material (Charkhi et al. 2010).

**Preparation of ZnCaNPs**

NaOH (1.11 g) was dissolved in purified water, and 5 g of caffeic acid was added. Moreover, the reaction mixture was kept under stirring on a hot plate to form sodium caffeinate. ZnCl\(_2\) (3.78 g) was dissolved in distilled water and followed by sodium caffeinate was added to form yellow precipitate from zinc caffeinate, separated by filtering and washing to dry in the oven. Zinc caffeinate powder was ground in the ball mill to convert into nanoscale material (Charkhi et al. 2010).

**Verification of ZnCoNPs and ZnCaNPs**

The structure of the prepared nanosystem was characterized by a vibrating sample magnetometer (VSM), transmission electron microscopy (HRTEM), Fourier transform infrared spectroscopy (FTIR), and wide range X-ray diffraction (WAXD).

**Determination of body weight and morphological state**

Body weight changes and morphological state had been recorded for all rats throughout the experimental period using the equation:

\[
\text{Percent of change} = \frac{\text{wt at week}(n) - \text{wt at week}(0)}{\text{wt at week}(0)} \times 100.
\]

**Complete blood count (CBC) determination**

Complete blood count was estimated by Sachse and Henkel (1996). Moreover, the performance of the CELL-DYN 1700 (Abbott Diagnostics, Abbott Park, IL, USA) is a multiparameter, mechanical hematology analyzer system.

**Antioxidant and oxidant markers analysis**

Superoxide dismutase activity (SOD), glutathione peroxidase activity (GPx), and catalase (CAT), and glutathione content (GSH) were measured in the blood using the method of Minami and Yoshikawa (1979) Paglia and Valentine (1967), Aebi (1984), and Beutler et al. (1963), respectively. Determination of antioxidant molecules and enzymes was done via commercial kits (Biodiagnostic, Giza, Egypt). Lipid peroxidation is measured calorimetrically as described by Yoshioka et al. (1979). Furthermore, nitric oxide (NOx) content as nitrite concentration was determined according to the method of Miranda et al. (2001), using commercial kits (Biodiagnostic, Egypt). Total free radicals were determined by the electron paramagnetic resonance technique (EPR) according to Heckly (1975).

**ROS detection by EPR**

The EPR measurements were carried with an EMX X-band spectrometer (Model E2-041XG, Bruker, Biospin, Germany) equipped with a standard rectangular ER 4102 cavity. All samples were analyzed at the same operating parameters, as follows: microwave power, 5.053 mW; modulation frequency, 100.0 kHz; modulation amplitude, 4.0 Gauss; sweep width, 150.0 Gauss; microwave frequency, 9.7 GHz; receiver gain 7.96 \( \times 10^{3} \); and central field, 3449.18 Gauss.

For calibration of the EPR measurement during the analysis procedure, a reference material, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was investigated in the EPR spectrometer before and after the analysis of samples under conditions similar to those of the sample measurements to minimize the errors resulting from the instability of the EPR spectrometer. The obtained EPR signal height for the samples (SHs) was normalized to the signal height of the reference (SHr) and the individual mass of the sample as follows:

\[
\text{Normalized EPR response} = \frac{\text{SHs}}{\text{SHr}} \times m,
\]

where \( m \) is the mass of the samples in mg.

Blood samples were dehydrated in absolute alcohol by immersion for 10 min (five samples/ group). These materials and sediment were then oven-dried at 40°C. After drying, the samples were carefully crushed with agate
mortal and pestle for homogenization under low impact, to avoid inducing other radicals by mechanical action. Subsequently, an aliquot of each sample was transferred to an EPR quartz tube, with a 3-mm internal diameter (Leite et al. 2018). The extraction of a-phenyl-N-tert-butyl nitrone spin adduct in ethyl acetate to increase its half-life time and detect its EPR has been described in previous reports in animal models of human blood. Ethyl acetate extracts were transferred to a flat quartz cell, and the spectrum was recorded (Facorro et al. 2004).

Determination of DNA damage in liver tissue by comet assay

Glass slides were coated with a thin layer of agarose, (35 µL) at 0.5% normal melting point(Sigma, St. Louis, MO, USA). The coating solution was placed on a glass slide and dried with an alcohol lamp (Changshin Scientific Co., South Korea). The slide was again covered with 75 µL of 0.5% NMA solution by diffusion with a slip cap and stored for 5 min in an ice bath to enhance gel formation.

A mixture of 100-µL cell suspension and 1% low melting point agarose (50 µL:100µL mixed at 45 °C) was placed on pre-chip wrappers, applied in layers by spreading with a sliding cover, and left the gel in frozen water for 5 min. Slides were submerged in neutral lysis buffer (0.2% SDS, 40mMTris-acetate, 1 m M EDTA and pH 8.0) for 30 min at room temperature and rinsed by immersion in electrical electrophoresis solution [Tris–borate-EDTA (TBE)] (45mMTris–borate, 1 mM EDTA and pH 8.4). Moreover, slides were electrocuted at room temperature at 2 V, 300 mA for 2 min in a horizontal electrophoresis chamber (Hoefer, Inc, San Francisco, CA, USA) saturated with TBE. The slides were then immersed in distilled water for 5 min and air dried and treated with 100 µL of the fluorescent pigment (YOYO-1; 10 µg mL–1, Molecular Probes, Eugene, OR, USA), fixed with a cover glass and dried for 20 min (Bhatti et al. 2012). The stained slide was shown under fluorescent (Optical Microscope Olympus, Tokyo, Japan) at a magnification shown under fluorescent (Optical Microscope Olympus, Tokyo, Japan) at a magnification x250 filters with exciting green. The images were transported using a charge-coupled video camera to a computer and analyzed, using the Comet Assay II image analysis system (Perceptive Instruments, Suffolk, UK) to estimate DNA migration. The tail length and the tail moment were measured as the degree of DNA damage. Two hundred cells were arbitrarily selected and calculated (two slides made for one sample, 100 randomly selected cells per slide) from one sample. Statistical measurements were performed using the Excel 5.0 program (Microsoft, Redmond, USA), which formed the standards of the mean tail length for a sample and the values of percentage cells in six ranges of tail length.

Flow cytometry assays

In addition to phosphate-buffered saline buffer, 1 × 10⁶ cell/mL was produced by isolating mononuclear cells from the blood with Ficoll-Paque. Moreover, 100 µL of cell suspension was added to propidum iodide (PI with RNase) buffer and kept for at least 1 h in the dark at +4 °C. The cells were obtained by BD Flow Cytometry Accurc C6Plus (Becton Dickinson, San Diego, CA) after incubation. The DNA of 10,000 cells was analyzed by fluorescence-activated cell sorter (FACS) Caliber Flow Cytometry to estimate the percentages of cells (El Tawiil et al. 2020). Cell surface antigen expression was examined by FACS analysis with monoclonal antibody (mAbs). FITC-conjugated anti-CD4, FITC-conjugated anti-CD8, and FITC-conjugated mouse isotype matched control mAbs were from BD Biosciences, Franklin Lakes, NJ, USA (Guida et al. 2016). Samples were repeated thrice.

Histopathological study

Liver samples were taken and placed in a 10% buffered formalin fixation and then dehydrated, cleared and embedded in paraffin. Moreover, 5-µm tissues were prepared and stained regularly with hematoxylin and eosin following Bancroft and Stevens (1996) and microscopically investigated.

Statistical analysis

All the standards are considered as mean ± standard deviation (SD). Experimental data were analyzed via one-way analysis of variance (ANOVA) and Tukey’s post hoc test to assess the significant variations between the means. The significance levels were p < 0.05, p < 0.01, and p < 0.001.

Results

From the transmission electron microscopy (TEM) results, Fig. 1 represents TEM image of the ZnCoNPs (Fig. 1a) ranging from 15 to 25 nm in diameter and have a spherical shape, while ZnCaNPs (Fig. 1b) range from 7 to 12 nm as shown in the TEM image and the magnification of image (20,000).

The FTIR spectra of p-coumaric acid and ZnCoNPs samples (Fig. 1C) are similar and show typical bands. The band derived from stretching hydroxyl group vibrations ν(OH) is located at 3385 cm⁻¹. Bands assigned to C=C stretches of the double bond are at 1638 cm⁻¹. Peak characteristics for the aromatic ring are 1606–1428 cm⁻¹ and bands assigned to in-plane CH deformations are present at 1255–1106 cm⁻¹. At lower wave numbers, peaks derived from out-of-plane CH deformations are located at 974–829 cm⁻¹. Peaks assigned to carboxylate vibrations are νs (COO−; 1,512 cm⁻¹) and νs
Fig. 1 Transmission electron microscope image of the ZnCoNPs (a) and ZnCaNPs (b) with magnification of 20,000; the ZnCoNPs (a) are ranging from 15 to 25 nm in diameter and have a spherical shape, while ZnCaNPs (b) range from 7 to 12 nm. FTIR spectrum of p-coumaric acid and ZnCoNPs (c) and caffeic acid and ZnCaNPs (d). XRD patterns of p-coumaric acid, ZnCoNPs, and ZnCaNPs (e).
(COO⁻; 1,400 cm⁻¹). The FTIR spectroscopic data approve the band integrity at 1,673 cm⁻¹ in the FTR spectrum of p-coumaric acid, which disappears with deprotonation, observed in the ZnCoNPs spectrum. Moreover, the band at
940 cm\(^{-1}\) can be assigned to the OH bending of the carboxyl group which only appears in the p-coumaric acid spectrum.

FTIR spectrum of caffeic acid and ZnCaNPs (Fig. 1D), which appear in the 4,000–2,600 cm\(^{-1}\) region, were assigned to the different OH vibrations modes. The high-frequency region is also characterized by the weak CH stretching modes of benzene moiety and acyclic chain. The vibrational contributions to the normal stretching modes in the 3,428–3,231 cm\(^{-1}\) region are assigned almost solely to the OH and CH stretching modes themselves. The bands of the strong intensities at 1,645 were assigned to the CO stretching modes of the carboxyl group. In addition, the intensity bands in the IR spectrum at 1,616 cm\(^{-1}\) assigned to C = C, and 1,450 cm\(^{-1}\) were mostly assigned to the CC stretching modes of both benzene moiety and acyclic chains. There was a slight shift due to ZnCaNPs formation.

XRD patterns of p-coumaric acid, ZnCoNPs, and ZnCaNPs are shown in (Fig. 1E). The main values of 2\(\theta\)/\(^\circ\) of the p-coumaric acid peaks beside new peaks appeared at 2\(\theta\)/\(^\circ\); 15.8\(^\circ\), 16.99\(^\circ\), and 27.7\(^\circ\) for zinc in ZnCoNPs. These peaks also appeared in XRD patterns of ZnCaNPs which confirmed their crystal structures.

Table 1 and Fig. 2 showed the percentage change in body weight and the morphological state of the animals. The percentage change in the body weight of the animals exposed to \(\gamma\)-rays 7 Gy was significantly reduced \((p \leq 0.001)\) and the morphological state deteriorated compared to control animals.

Moreover, the 7-day ZnCoNPs + ZnCaNPs treatment in normal rats had no alterations \((p \geq 0.05)\) in body weight and morphological state, compared with the parallel control values. However, irradiated rats treated with ZnCoNPs + ZnCaNPs for 7 days revealed a noticeable augment \((p \leq 0.001)\) in the percentage change of the bodyweight and improved morphological state, compared with the irradiated rats.

In the present work, our data demonstrated that the complete blood count (CBC) level in irradiated rats had a highly significant decrease \((p \leq 0.001)\) in the value of white blood cells (WBCs) and a WBC differential which are lymphocytes \([\text{LYM (RM), LYM (%)}]\), monocyte \([\text{MID, MID % M}]\), and granulocytes \([\text{GRAN (RM), GRAN (G %)}]\) (Table 2).

Radiation effect on red blood cells (RBCs) count are shown in Table (2); our data showed a highly significant decrease \((p \leq 0.001)\) in the value of RBCs. Additionally, a significant diminish \((p \leq 0.001)\) in the value of hemoglobin (HGB) and hematocrit (HCT) was observed compared with the control (Table 2). Moreover, a marked reduction \((p \leq 0.001)\) in the value of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), platelet count test (PLT) is associated with a significant elevation \((p \leq 0.05)\) in the value of mean

### Table 1

| Groups Parameters | Control | ZnCoNPs + ZnCaNPs | IR | IR + ZnCoNPs + ZnCaNPs |
|-------------------|---------|-------------------|----|------------------------|
| N                 | 8       | 8                 | 8  | 8                      |
| D                 | —       | —                 | 4  | —                      |
| Body weight       | 129±16  | 137±3.66          | 114±1.8 \(^{a1}\) | 136±1.8 \(^{b3}\) |
|                   |         |                   | (-12) |                      |

All values are expressed as means ± SD \((n=8)\). Values between brackets show the percentage of change from control. Values marked with letters are significantly different \((a1: p \leq 0.05 \text{ [significance vs. control group]; } b3: p \leq 0.001 \text{ [significance vs. irradiated group (IR)]})\). N: Number of live rats. D: Number of dead rats.

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**Fig. 2** Effect of ZnCoNPs + ZnCaNPs and/or IR on the morphological state (a) and DNA fragmentation in live tissues (b) in the different animal groups.
corpuscular hemoglobin concentration (MCHC), compared to the control normal group (Table 2).

On the other hand, the effect of ZnCoNPs + ZnCaNPs treatment for 7 days to normal rats had no marked alterations \( (p \geq 0.05) \) in the level of complete blood count, compared to their parallel control values, while irradiated rats treated with ZnCoNPs + ZnCaNPs for 7 days revealed a marked positive modulation in the complete blood count (CBC), compared to the irradiated one.

Data showed that the WBCs and a WBC differential which are lymphocyte [LYM (RM), LYM (%)], monocyte (MID, MID % M), granulocytes [GRAN (RM), GRAN (G %)] as well as RBCs would exhibit significant \( (p < 0.001) \) increases (Table 2).

Moreover, a significant elevation \( (p \leq 0.001) \) in the value of HGB and HCT% was noticed compared with their values of irradiated rats (Table 2). Besides, a marked augment \( (p \leq 0.001) \) in the MCV, MCH, RDW, and PLT is associated with an obvious drop \( (p \leq 0.05) \) in the value of MCHC, compared to the irradiated group (Table 2).

The standard alkaline comet assay results, presented in (Table 3) and (Fig. 2), show the DNA damage manifested by a significant increase in the level of tail length, DNA in the tail, tail moment, and olive tail moment, compared with values in control rats. Furthermore, in normal rats, ZnCoNPs + ZnCaNPs treatment showed a significant decrease in the level of tail length, DNA in the tail, tail moment, and olive tail moment, compared with values in the control rats. Moreover, ZnCoNPs + ZnCaNPs treatment

### Table 2 Effect of ZnCoNPs + ZnCaNPs and/or IR on the complete blood count level in the different groups

| Parameters     | Control          | ZnCoNPs + ZnCaNPs | IR               | IR + ZnCoNPs + ZnCaNPs |
|----------------|------------------|-------------------|------------------|------------------------|
| WBC (K/uL)     | 8.7 ± 0.2        | 8.2 ± 0.2         | 1.3 ± 0.2\textsuperscript{a1} | 7.4 ± 0.0\textsuperscript{b3} |
|                | (−6)             | (−5)              | (−15)            | (−15)                  |
| LYM (RM)       | 2.9 ± 0.5        | 2.8 ± 0.1         | 0.5 ± 0.01\textsuperscript{a3} | 2.3 ± 0.5\textsuperscript{b3} |
|                | (−3)             | (−10)             | (−20)            | (−20)                  |
| LYM (%)        | 44 ± 3.9         | 45.2 ± 4.9        | 10 ± 1.2\textsuperscript{a3} | 43 ± 4.3\textsuperscript{b3} |
|                | (3)              | (−77)             | (−2)             | (−2)                   |
| MID (Monocyte) | 0.80 ± 0.0       | 0.77 ± 0.0        | 0.2 ± 0.01\textsuperscript{a3} | 0.70 ± 0.05\textsuperscript{b3} |
|                | 0                | (−75)             | (−13)            | (−13)                  |
| MID (Monocyte %M) | 6.9 ± 0.0       | 6.9 ± 0.0         | 1.3 ± 0.2\textsuperscript{a3} | 6.8 ± 1.9\textsuperscript{b3} |
|                | 0                | (−81)             | (−1)             | (−1)                   |
| GRAN (RM)      | 4 ± 1.4          | 3.7 ± 1.3         | 0.5 ± 0.6\textsuperscript{a3} | 3.0 ± 1.1\textsuperscript{b3} |
|                | (−8)             | (−87)             | (−25)            | (−25)                  |
| GRAN (%G)      | 50.7 ± 5.2       | 52.4 ± 7.5        | 2 ± 0.1\textsuperscript{a3} | 40.2 ± 4.1\textsuperscript{b3} |
|                | (3)              | (−96)             | (11)             | (11)                   |
| RBC (M/uL)     | 5.2 ± 0.08       | 5.4 ± 0.08        | 3 ± 0.05\textsuperscript{a3} | 4.5 ± 0.09\textsuperscript{b3} |
|                | (4)              | (−42)             | (−13)            | (−13)                  |
| HGB (g/dL)     | 13.7 ± 0.7       | 13.6 ± 0.8        | 4.6 ± 0.8\textsuperscript{a3} | 12.9 ± 2.6\textsuperscript{b3} |
|                | (−0.7)           | (−66)             | (−6)             | (−6)                   |
| HCT %          | 41.6 ± 3.8       | 42.4 ± 3.7        | 18.8 ± 1.7\textsuperscript{a3} | 41.1 ± 3.7\textsuperscript{b3} |
|                | (2)              | (−55)             | (−1)             | (−1)                   |
| MCV (fL)       | 89 ± 7           | 88 ± 8            | 48 ± 4.9\textsuperscript{a3} | 80 ± 8\textsuperscript{b3} |
|                | (0.1)            | (−46)             | (−10)            | (−10)                  |
| MCH (pg)       | 29.3 ± 2.7       | 28.9 ± 0.7        | 15.2 ± 1.8\textsuperscript{a3} | 25.1 ± 1.6\textsuperscript{b3} |
|                | (−1)             | (−48)             | (−14)            | (−14)                  |
| MCHC (g/dL)    | 33.6 ± 2.4       | 33.4 ± 0.3        | 38.9 ± 1.6\textsuperscript{a3} | 33.8 ± 2.4\textsuperscript{b3} |
|                | (−1)             | (16)              | (1)              | (1)                    |
| RDW %          | 13 ± 1.1         | 12.7 ± 0.7        | 4.1 ± 0.7\textsuperscript{a3} | 12.6 ± 1.8\textsuperscript{b3} |
|                | (−2)             | (−69)             | (−3)             | (−3)                   |
| PLT (K/uL)     | 293 ± 57         | 294 ± 49          | 111 ± 23\textsuperscript{a3} | 291 ± 50\textsuperscript{b3} |
|                | (0.2)            | (−61)             | (−0.5)           | (−0.5)                  |

All values are expressed as means ± SD \( (n = 8) \). Values between brackets show the percentage of change from control. Values marked with letters are significantly different \( (a1, p \leq 0.05; a2, p \leq 0.01; a3, p \leq 0.001 [significance vs. control group]; b1, p \leq 0.05; b2, p \leq 0.01; b3, p \leq 0.001 [significance vs. irradiated group (IR)]) \)

\( ≤ \)
in irradiated rats confirmed DNA repair as evidenced by a significant decrease in the tail length, percentage of DNA in the tail, tail moment, and olive tail moment, compared with their corresponding values in the irradiated one.

Table 4 showed a remarkable reduction \((p \leq 0.001)\) in the activity of SOD, GSHpx, and CAT and GSH contents, associated with a significant elevation \((p \leq 0.001)\) in the values of NOx, LP, and TFRC in the irradiated group, compared with the normal group.

Treatment with ZnCoNPs + ZnCaNPs for 7 days in normal rats had no changes in the level of antioxidant enzymes and content. Moreover, the activity of SOD, GSHpx, CAT and GSH content displayed an obvious increase \((p \leq 0.001)\) linked with a major reduction \((p \leq 0.001)\) in the level of NOx, LP, and total FRC (Fig. 3) in the irradiated rats treated with ZnCoNPs + ZnCaNPs, compared to irradiated rats.

Table 5 and Fig. 4 show the lymphocyte population’s data for CD4 T helper and CD8 T cytotoxic cells in the blood. The post hoc analysis showed a significant decrease \((p \leq 0.001)\) in irradiated rats, compared with normal control.

Moreover, animals injected with ZnCoNPs + ZnCaNPs showed no significant changes \((p \geq 0.001)\) in the lymphocyte population data, compared with the irradiated group, while irradiated rats injected with ZnCoNPs + ZnCaNPs showed amelioration in the lymphocyte population data that appeared as a noticeable increase in the percentage of CD4 and CD8 (Table 5 and Fig. 4), compared with the irradiated group.

Additionally, the result in the irradiated group decreased significantly \((p \leq 0.001)\) in the S phase and

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### Table 3

Different effects of ZnCoNPs + ZnCaNPs and/or IR on DNA fragmentation level in liver tissues in the different groups

| Groups Parameter | Control | ZnCoNPs + ZnCaNPs | IR | IR + ZnCoNPs + ZnCaNPs |
|------------------|---------|-------------------|----|------------------------|
| Tail length μm   | 0.3 ± 0.6 | 84.5 ± 4.5**      | 0.3 ± 0.6 |                       |
| DNA in tail      | 0.8 ± 1.3 | 3.1 ± 1.3**      | 0.04 ± 0.004 |                       |
| Tail moment      | 0        | 2.5 ± 1**       | 0        |                       |
| Olive moment     | 0.007 ± 0.1 | 1.4 ± 0.7**   | 0        |                       |

All values are expressed as means ± SD \((n = 8)\). Values between brackets show the percentage of change from control. Values marked with letters are significantly different \((a1, p \leq 0.05; a2, p \leq 0.01; a3, p \leq 0.001)\) [significance vs. control group]; b1, \(p \leq 0.05\); b2, \(p \leq 0.01\); b3, \(p \leq 0.001\) [significance vs. irradiated group (IR)].

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### Table 4

Effects of ZnCoNPs + ZnCaNPs and/or IR on the activity of superoxide dismutase, glutathione peroxidase, catalase, glutathione content, lipid peroxidation, total free radical concentrate, and total nitric oxide in different groups

| Groups Parameters | Control | ZnCoNPs + ZnCaNPs | IR | IR + ZnCoNPs + ZnCaNPs |
|------------------|---------|-------------------|----|------------------------|
| SOD (μg/mL)      | 4.3 ± 1.3 | 5.4 ± 0.4**      | 0.9 ± 0.3** | 3.8 ± 0.7** |
| GSH (mg/mol)     | 38.5 ± 4 | 41.4 ± 3.1**    | 10.8 ± 1.6** | 39.1 ± 3.9** |
| GPx (min/mL)     | 7.1 ± 1.2 | 7.5 ± 0.2**     | 1.9 ± 0.1** | 6.2 ± 0.2** |
| CAT (μmol/mL)    | 5.8 ± 1.1 | 7.1 ± 0.2**     | 4 ± 0.2**   | 6 ± 0.5** |
| LP (mM/L)        | 1.7 ± 0.2 | 1.6 ± 0.1       | 3 ± 0.09a | 1.9 ± 0.1a |
| NOx (μmol/L)     | 23.6 ± 2.9 | 20.9 ± 2.3**   | 36.3 ± 3.2** | 28.7 ± 3.9** |
| TFRC(Radicals/g) | 0.48 ± 0.01 | 0.44 ± 0.01   | 4.1 ± 0.3** | 0.47 ± 0.02** |

All values are expressed as means ± SD \((n = 8)\). Values between brackets show the percentage of change from control. Values marked with letters are significantly different \((a1, p \leq 0.05; a2, p \leq 0.01; a3, p \leq 0.001)\) [significance vs. control group]; b1, \(p \leq 0.05\); b2, \(p \leq 0.01\); b3, \(p \leq 0.001\) [significance vs. irradiated group (IR)].
increased visibly ($p \leq 0.001$) in the sub G1 (apoptosis), G0/1, and G2/M phases compared with the normal group (Table 6).

The treatment with ZnCoNPs + ZnCaNPs in normal rats had no changes ($p \leq 0.001$) in the S, sub G1 (apoptosis), G0/1, and G2/M phases, compared with the normal group. Moreover, ZnCoNPs + ZnCaNPs injection to irradiated animals demonstrated a significant enhancement ($p \leq 0.001$) in the S phase and a remarkable decline ($p \leq 0.001$) in the sub G1 (apoptosis), G0/1, and G2/M phases (Table 6), compared with the irradiated group.

The liver of a control rat under a light microscope appeared to be composed of lobules with central veins and peripheral hepatic triads embedded in the connective tissues. The sides between the cell cords are designated by blood sinusoids and covered by Kupffer cells. The hepatocytes are regular and have a large spherical nucleus with nucleolus and peripheral chromatin distribution and binucleated cells were also appeared (Fig. 5a). Fig. 5b showed that normal rats treated with ZnCoNPs + ZnCaNPs revealed no significant change, but irradiated rats’ livers showed noticeable degenerative alterations in most liver cells. The cells were expanded and contained light and foamy cytoplasm packed with several vacuole-like areas. The walls of the blood sinusoids were dilated and showed abundant Kupffer cells. The γ-irradiation also revealed signs of hepatocytes necrotic changes with pyknotic nuclei and hepatocyte degeneration with strong acidophilic cytoplasm. Diffused hemorrhagic areas and interstitial edema were observed (Fig. 5c). No pathological changes in Irradiated + ZnCoNPs + ZnCaNPs group were noted, which showed a normal lobular manner with central vein and radiating hepatic cords (Fig. 5d).

**Discussion**

Radiation motivates a remarkable change in the metabolic actions series resulting in oxidative stress involved in the process of carcinogenesis and different diseases (Liguori et al. 2018; Yahyapour et al. 2018). The damage induced by radiation in the biological organs may be due to the indirect impact of radiation on DNA, RNA, proteins, and lipids or the indirect impact caused by water radiolysis and excess ROS generation that confirmed the mechanisms of IR-induced cytotoxicity (Khan et al. 2018).

Data from the current study exhibited that irradiated rats treated with ZnCoNPs in combination with ZnCaNPs showed a marked improvement in the liver manifested by a positive modulation in the percentage change of body weight and morphological state and oxidative and antioxidant markers, as well as in DNA damage, CD4, CD8, blood parameters, and cell cycle.

The results agree with the work of Takahashi et al. (2020) who reported a loss of body weight in rats post-irradiation. However, ZnCoNPs combined with ZnCaNPs, a natural nanoprotector, are considered a targeting mediator for preventing free radical damage due to their effect on catabolic radiation.

Irradiation induces direct disorder of the circulatory system resulting in hemorrhage and diminishes cell formation (Hamada et al. 2020). The present study observed a decreased level of hematological parameters in the irradiated rats indicating anemia. Moreover, the reduction could be caused by direct injury induced by a lethal dosage of γ-rays (Bala et al. 2019). The cellular blood parameters are mainly susceptible to oxidative stress. A high ratio of polyunsaturated fatty acids in their membranes induces membrane LP, leading to membrane rigidity, cellular deformability, and reduced erythrocyte survival reduction (Adams et al. 2015).

Moreover, the reduction in the hemoglobin content may be caused by the decreased number of RBCs and hemorrhage. Additionally, the decrease in the hematocrit value might result from the decrease in erythropoietin, destruction of mature cells, or increased plasma volume. Furthermore, the lowering in the values of blood components after γ-rays exposure may be due to a bone marrow syndrome (Green and Rubin 2014; Taqi et al. 2019).

Following the injection of ZnCoNPs and ZnCaNPs in irradiated rats, the alterations of hematological parameters may be attributable to its free radical scavenging and RBCs. Thus, its related indices improved. Moreover, the result may occur via stimulating formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow of rats to produce new RBCs (Choi et al. 2019; Kianmehr et al. 2020).

The current study recorded a drop in the activity of SOD, GPx, CAT, and GSH contents, which may be due to radiation-induced impairment in the antioxidant defense mechanism. In addition, the decreased antioxidant levels may be due to their utilization by the enhanced ROS increase (Prasad et al. 2005; Kurutas 2016).

The antioxidant response element mediates the transcriptional activation of several gene upstream of the many phase II detoxification and antioxidant enzymes in response to oxidative stress. Furthermore, SOD, GPx, and CAT genes are considered regulated with the respective antioxidant response elements (Saha et al. 2020). Thus, the current study suggests that ZnCoNPs in combination with ZnCaNPs are transcriptional agents and stimulates the gene expression during cell oxidation resulting in an augment in the synthesis of the antioxidant enzymes (e.g., blood SOD, GSH, GPx, and CAT) (Kanagaraj et al. 2015). Moreover, p-coumaric and caffeic acids can stimulate the activity of phase II detoxification enzymes and oxidative...
Fig. 3 (a) Effect of ZnCoNPs + ZnCaNPs and/or IR on the level of total free radicals in the different groups, \( \frac{1}{m} \frac{d^2x}{dh} \) (arb.units), where \( m \) is the mass, \( dx \) is the 2nd derivative, \( dh \) is the absorbed intensity of magnetic flux, and (arb) is the arbitrary unit. (b) Bruker EMX EPR spectrometer located in the National Center for Radiation Research and Technology (NCRRT)
Table 5 Different effects of ZnCoNPs + ZnCaNPs and/or IR on T helper cells (CD4) and T cytotoxic cells (CD8) expression in the different groups

| Groups          | Control | ZnCoNPs + ZnCaNPs | IR       | IR + ZnCoNPs + ZnCaNPs |
|-----------------|---------|-------------------|----------|-------------------------|
| Parameter       |         |                   |          |                         |
| CD4             | 49.9 ± 4.8 | 50 ± 1.9          | 18.2 ± 1.3³ | 40.9 ± 3.8³b³           |
|                 | (0.2)     | (−64)             | (−18)    |                         |
| CD8             | 12.5 ± 1.9 | 13.8 ± 3.4        | 4.5 ± 0.4³ | 10.5 ± 2.2³b³           |
|                 | (10)      | (−64)             | (−16)    |                         |

All values are expressed as means ± SD (n = 8). Values between brackets show the percentage of change from control. Values marked with letters are significantly different (a1, p ≤ 0.05; a2, p ≤ 0.01; a3, p ≤ 0.001 [significance vs. control group]; b1, p ≤ 0.05; b2, p ≤ 0.01; b3, p ≤ 0.001 [significance vs. irradiated group (IR)]

Fig. 4 Effect of ZnCoNPs + ZnCaNPs and/or IR on the cell cycle phases (a) and expression of T helper cells, CD4 and T cytotoxic cells, and CD8 (b) in the different groups. Samples were analysis triplicate. Flow cytometric images of cell cycle, CD4, and CD8. The results presented as the mean ± SEM, n = 8.
stress inhibition (Chen et al. 2020). The location of the hydroxyl groups in the ZnCoNPs and ZnCaNPs may share in the stimulation of \( \gamma \)-glutamyl cysteine synthase, which is the rate-limiting enzyme implicated in glutathione production (Lee et al. 2016).

High concentration levels of nitric oxide (NO) have a mutual negative relationship with tissue function and are implicated in diverse physiological and pathological functions (de Oliveira et al. 2017). Endothelial nitric oxide synthase, neuronal NOS, and inducible NOS are dependent on calmodulin binding and are affected by calcium levels in the cells (Weissman et al. 2002). Thereby, the current study suggests that increased calcium levels due to radiation exposure (Nuszkiewicz et al. 2020) may lead to increased calmodulin binding to NOS isoforms, resulting in a transient increase in NO production by these enzymes (Silva and Ballejo 2019). Moreover, \( \gamma \)-rays may increase the endogenous NO production by direct DNA impairment, which motivates poly ADP-ribose polymerase that in turn excite the activation of nuclear factor kappa B to end with an increase of the iNOS expression and NO formation (Wilson et al. 2019). Moreover, ZnCoNPs and ZnCaNPs treatment post radiation was established to diminish LP and NOx that could be attributable to their cysteine source, which can stimulate GSH synthesis that protects the body against cell oxidation (Smith et al. 2017).

This study demonstrated that \( \gamma \)-rays induced an excess of free radicals that can produce DNA damage and permanent impairment, signal transduction pathway alterations, and cytogenetic and biochemical defects (Prasad et al. 2005). Thus, DNA impairment may be attributable to an increased concentration of vasoconstrictors or due to the decline of vasodilators (i.e., NO). Moreover, the data in the current study showed that ZnCoNPs in combination with ZnCaNPs could mitigate the DNA damage or fragmentation against oxidative damage induced by singlet oxygen (Reza et al. 2016). These findings are consistent with Srinivasan et al. who reported that curcumin is a part of the natural compound ZnCoNPs and ZnCaNPs, which protect the DNA by reducing chromosomal aberrations (Srinivasan et al. 2007).

The impaired immunological function may result from immune system impairment. The immune system response may be due to the action of T cells lymphocytes originated in the thymus. T lymphocytes comprise the cytotoxic T cells (CD8), which respond to cells and are affected by viruses or tumor cells; T helper cells (CD4) that produce mediators to stimulate lymphocytes; B cells; macrophages; natural killer cells; and the T cells themselves (Li et al. 2015).

Herein, exposure to IR causes the lymphocytes to die via apoptosis. Moreover, a decrease in the CD4 and CD8 level occurred in the current work, and this follows (Li et al. 2015). The data of the current study displayed that irradiated rats treated with ZnCoNPs and ZnCaNPs protect CD4 and CD8 from the hazard of \( \gamma \)-ray, which is similar to Pragasam et al. (2013) and Choi et al. (2019).

Irradiated rats in this study showed a high level of cell death induction, where the number of cells in the G0/1 and S phases and the cell cycle decreased markedly. However, the cells in the sub-G1 (apoptosis) and G2/M phases increased significantly. This result may be caused by apoptosis induction (Vucić et al. 2006; Durante and Formenti 2018).

The results showed that the treatment with ZnCoNPs and ZnCaNPs post-radiation mitigated the cell cycle phases that resulted from the risk of \( \gamma \)-ray, which agrees with Rosa et al. (2018).

Moreover, the current study’s data indicated that the liver of the rats exposed to \( \gamma \)-rays resulted in diverse lesions with dilated blood vessels and pyknosis, hemorrhage, vacuolation, and necrobiotic alterations in the hepatocytes. Zavodnik et al. (2003) and Guryev (2005) found similar results. Additionally, the tissue of irradiated rats showed fibrosis, the most common delayed effect of

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### Table 6: Different effects of ZnCoNPs + ZnCaNPs and/or IR on cell cycle phases in the different groups

| Groups          | Parameter | Control | ZnCoNPs + ZnCaNPs | IR | IR + ZnCoNPs + ZnCaNPs |
|-----------------|-----------|---------|-------------------|----|------------------------|
|                 | Sub G1(Apoptosis) | 41.3 ± 4.6 | 23 ± 3.6<sup>a1</sup> | 8.2 ± 1<sup>a1</sup> | 33.4 ± 4.2<sup>b1</sup> |
|                 | G0/1      | 38 ± 3.3 | 43.7 ± 3.8<sup>a1</sup> | 8.1 ± 0.8<sup>a1</sup> | 37.4 ± 4.7<sup>b3</sup> |
|                 | S%        | 11.1 ± 1.0 | 21.8 ± 3.1<sup>a3</sup> | 0.5 ± 0.07<sup>a3</sup> | 18.2 ± 2.1<sup>b3</sup> |
|                 | G2/M%     | 4.2 ± 0.3 | 4.0 ± 1.2<sup>a3</sup> | 13.6 ± 2.7<sup>a3</sup> | 3.6 ± 1<sup>b1</sup> |

All values are expressed as means ± SD (n = 8). Values between brackets show the percentage of change from control. Values marked with letters are significantly different (a1, p ≤ 0.05; a2, p ≤ 0.01; a3, p ≤ 0.001 [significance vs. control group]; b1, p ≤ 0.05; b2, p ≤ 0.01; b3, p ≤ 0.001 [significance vs. irradiated group (IR)]).
radiation; lack of parenchymal hepatocytes; and deformation of the lobular architecture associated with both per-central and per-portal fibrosis (Yıldızhan et al. 2020).

Irradiated rats treated with ZnCoNPs and ZnCaNPs could attenuate the adverse effects of γ-radiation exposure and similar results reported by Pang et al. (2016). The mitigating impact of ZnCoNPs and ZnCaNPs against hepatotoxicity may depend on the antioxidant effects (Ali and Zeyadi 2020).

Conclusion

The present study suggests that ZnCoNPs in combination with ZnCaNPs could be used as radiomitigators to treat harmed hepatic tissues induced by radiation. The treatment of irradiated rats showed a positive modulation in the marker of antioxidant and oxidant parameters, blood elements, DNA, CD4, CD8, and cell cycle phases. Additionally, the results were confirmed by a histopathological study, which exhibited an improvement in the liver tissues of rats.

Authors’ contributions

All authors planned the experiment. Askar MA, Mansour NA, Ali EN, Abdel-Magied N, and Ragab EA prepared the samples of tissues and nanoparticles. Guida MS prepared the samples of electrophoresis. Abu Nour SM prepared the samples for histopathological study. Elmasry SA prepared the samples for Flow Cytometry study. All authors analyzed the data. All authors read and approved the final manuscript.

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Data availability

Data are available on request to the authors.

Fig. 5 Effect of ZnCoNPs + ZnCaNPs and/or IR on histopathological changes of hepatocytes. (a) Light photomicrography of liver of a control rat showing the hepatocytes (H) are arranged into hepatic cords running radiantly from the central vein (CV) and are separated by adjacent blood sinusoids (S) containing Kupffer cells. (b) Liver of rats treated with ZnCoNPs + ZnCaNPs alone showing normal appearance of liver lobular architecture with central vein (CV).

(e) Liver of an irradiated rat showing the cellular structure of the liver tissues. The hepatocyte cytoplasm is light and foamy and filled with vacuoles; cell sizes are enlarged; and nuclear chromatin is more condensed. Odema areas (*) were observed. (d) Liver of rat of IR + ZnCoNPs + ZnCaNPs group showing normal lobular architecture with central vein and radiating hepatic liver. H&E. ×400
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