

# In vitro evaluation of anticancer and antibacterial activities of cobalt oxide nanoparticles

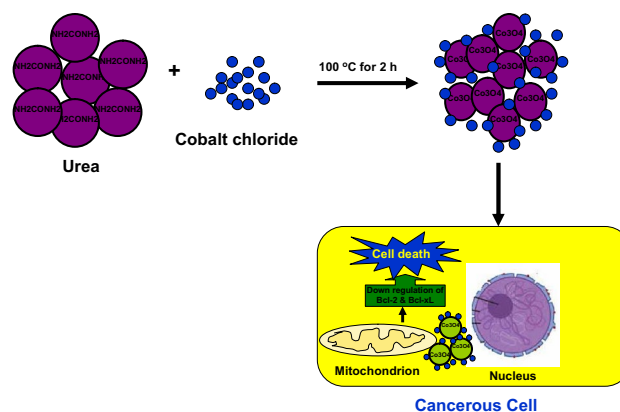
Shahanavaj Khan<sup>1,2</sup> · Anees A. Ansari<sup>3</sup> · Abdul Arif Khan<sup>1</sup> · Rehan Ahmad<sup>4</sup> · Omar Al-Obaid<sup>4</sup> · Wael Al-Kattan<sup>5</sup>

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**Abstract** Cobalt oxide nanoparticles ( $\text{Co}_3\text{O}_4$ -NPs) were synthesized using simple urea-based thermal decomposition method. Phase purity and particle size of as-synthesized nanoparticles were characterized through X-ray diffraction pattern (XRD) and transmission electron microscopy. Through XRD morphology of the  $\text{Co}_3\text{O}_4$ -NPs was found to be variable in size with range of 36 nm. In our present study, we explored the potential cytotoxic and antibacterial effects of  $\text{Co}_3\text{O}_4$ -NPs in human colorectal types of cancerous cells (HT29 and SW620) and also nine Gram-positive and Gram-negative bacteria.  $\text{Co}_3\text{O}_4$ -NPs showed promising anticancer activity against HT29 and SW620 cells with  $\text{IC}_{50}$  value of 2.26 and 394.5  $\mu\text{g}/\text{mL}$ , respectively. However, no significant effect of  $\text{Co}_3\text{O}_4$ -NPs was observed against bacterial strains. Furthermore, a detailed study has been carried out to investigate the possible mechanism of cell death in HT29 cancer cell line through the analysis of

expression level of anti-apoptotic Bcl2 and BclxL markers. Western blot analysis results suggested significant role of  $\text{Co}_3\text{O}_4$ -NPs exposure in cell death due to apoptosis.

## Graphical Abstract



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✉ Shahanavaj Khan  
khan.shahanavaj@gmail.com

- <sup>1</sup> Nanomedicine and Biotechnology Research Unit, Department of Pharmaceutics, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia
- <sup>2</sup> Department of Bioscience, Shri Ram Group of College (SRGC), Muzaffarnagar, UP, India
- <sup>3</sup> King Abdullah Institute for Nanotechnology, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
- <sup>4</sup> Colorectal Research Center, College of Medicine, King Saud University, Riyadh 11451, Saudi Arabia
- <sup>5</sup> Department of Surgery, Al-Faisal University Riyadh, Riyadh 11451, Saudi Arabia

**Keywords** Cobalt oxide nanoparticles · MTT assay · Cytotoxicity · Western blot analysis

## Introduction

Rapid development of variety of nanoparticles (NPs) attracts researchers due to its possible application in wide range of nano-biotechnological applications, particularly in advance biomedical purpose [1], drug carriers/delivery systems [2], and vaccine administration [3]. Cobalt is one of the elements of research interest, applied in the preparation of nanoparticles for biomedical applications [4]. Currently, cobalt oxide nanoparticles based on cobalt metal are able to attracting more interest of researchers due to their specific shape, size-dependent characteristics and also wide

applications [5]. In spite of its physiological role as a co-factor of vitamin B12, cobalt cannot only be regarded as an essential element.

Nanoparticles of metal oxide are extensively being utilized in various industrial products, i.e., catalysts, cosmetics, pigments, sun screens and food additives [6, 7]. Polymeric nanoparticles with functional properties have been widely used in a broad range of bio applications; like drug and gene delivery, cell and tissue engineering, diagnostic and therapeutic purposes, etc. [8–11]. Among these applications, the field of drug delivery by nanoparticles with specific and rapid internalization into a target cell has immense promise [12–15]. In vitro and in vivo experimental evidences showed that various metal oxide nanoparticles have potential toxicity [16–21]. Several metal NPs exhibited unique properties in terms of magnetic, electrical and optical activity [22]. In particular, cobalt, nickel and iron NPs are destined to discover their enigmatic role in medical nanotechnology and biotechnology due to their magnetic properties [23]. Various metal NPs, in fact, have been proposed for drug delivery and hyperthermic cancer treatment [24]. Furthermore, cobalt-based NPs generally, and cobalt oxide NPs ( $\text{Co}_3\text{O}_4$ -NPs) particularly, are attracting huge interest owing to their unique shape- and size-dependent properties and potential uses in catalysis, electrochemistry, sensors, pigments, magnetism, energy storage, etc. [25].

Focus of this work is to synthesize, characterize and develop economically viable and novel  $\text{Co}_3\text{O}_4$ -NPs which have significant biological potential. To define aggregation, size, shape and surface texture, we analyzed morphology of  $\text{Co}_3\text{O}_4$ -NP by XRD and electron microscopy. These properties may significantly influence their biological properties. In this study, we also evaluated toxicity of  $\text{Co}_3\text{O}_4$ -NPs on human cells; therefore, cellular viability was measured in HT29 and SW620 colon cancer cells and normal cells MCF-10A (non-cancerous epithelial cell line). HT29 and SW620 cells are commonly used as a surrogate for human colon cell line and have already discovered application in the study of nanoparticle cytotoxicity [26]. Furthermore, we also employed western blot analysis to evaluate the expression of anti-apoptotic markers to obtain new insights regarding the mechanisms of cytotoxicity and cell death into the cellular environment. In addition, we also employed disk diffusion method to evaluate the possible antibacterial activity in  $\text{Co}_3\text{O}_4$ -NP with the aim to obtain new insights regarding alteration in gut microbiome.

## Materials and methods

### Materials

Cobalt chloride (99.99 %, BDH Chemicals Ltd, England) and urea were used as starting materials without any

further purification. Urea, ethanol, dimethyl thiazolyltetrazolium bromide (MTT reagent), Doxorubicin hydrochloride (Control anti-cancerous drug), Fetal bovine serum (FBS) and  $\beta$ -actin, Bcl2, BclxL antibodies were purchased from Sigma Aldrich (St. Louis, MO, USA). RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM), 1 % antibiotic–antimycotic solution, Penicillin–streptomycin solution, and trypsin–EDTA solution were procured from Life Technologies GIBCO, Grand Island, NY, USA. HT29 and SW620 human colon cancer cell lines were purchased from American type cell culture collection (ATCC, Manassas, VA, USA). The ultrapure deionized water was used for the preparation of solutions. The ultrapure deionized water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). Remain all other reagents and chemicals used were of LR grade and purchased from Merck (Germany).

### Synthesis of $\text{Co}_3\text{O}_4$ -NPs

Cobalt chloride and urea were used as initial precursor for the synthesis of  $\text{Co}_3\text{O}_4$ -NPs, without any purification. 1.0 g cobalt chloride and 2 g urea were dissolved in 50 ml ultrapure distilled water separately. Furthermore, both solutions were mixed with constant stirring at 100 °C for 2 h. The resulting mixture was left from hot plate and separates the black color precipitate through centrifugation and washed several times with deionized water to remove the excess chloride and amines from the solution mixture. The prepared black precipitate was air dried at 100 °C and annealed at 300 °C for 2 h.

### Characterization of $\text{Co}_3\text{O}_4$ -NPs

#### *X-ray diffraction analysis of $\text{Co}_3\text{O}_4$ -NPs*

The crystallinity of the powder samples was examined by XRD at room temperature with the use of a PANalytical X'Pert X-ray diffractometer equipped with a Ni filter using Cu-K $\alpha$  ( $\lambda = 1.54056 \text{ \AA}$ ) radiation as the X-ray source. The average particle size of  $\text{Co}_3\text{O}_4$ -NPs was determined from the line width of the XRD peak.

#### *Analysis of $\text{Co}_3\text{O}_4$ -NPs by transmission electron microscopy (TEM)*

TEM analysis of  $\text{Co}_3\text{O}_4$ -NPs was performed on a transmission electron microscope (FE-TEM, JEM-2100F, JEOL, Japan) by operating at an accelerating voltage of 200 kV. Samples were prepared by depositing a drop of a colloidal ethanol solution of the powder sample of  $\text{Co}_3\text{O}_4$ -NPs (1 %  $\text{Co}_3\text{O}_4$ -NPs) onto carbon-coated gold TEM grids. Film of  $\text{Co}_3\text{O}_4$ -NPs sample on TEM grid was allowed to stand

for 3 min. The excess of solution was eliminated using a clean blotting paper and the grid was allowed to dry prior to measurement.

### Cell viability assays

Cell viability assays were performed to evaluate the anti-tumor potential of  $\text{Co}_3\text{O}_4$ -NPs using human colorectal cancer cell lines HT29 and SW620.

### Cell culture

Human colorectal cancer cells HT29, SW620 and normal cells MCF-10A (non-cancerous epithelial cell line) were obtained from American Type Culture Collection (ATCC). Initially, cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) or Roswell Park Memorial Institute (RPMI 1640) medium supplemented with 10 % fetal bovine serum and 100 U/mL penicillin, 100  $\mu\text{g}/\text{mL}$  streptomycin at 37 °C in incubator under 5 %  $\text{CO}_2$  and high humidity. At 75–85 % confluence, cells were harvested using 0.25 % trypsin by the process of trypsinization. Furthermore, the cells were subcultured into 75  $\text{cm}^2$  flasks and 96-well plates according to selection of experiments. Cells were allowed to attach to the surface for 24 h prior to treatment at 37 °C in 5 %  $\text{CO}_2$ . The serial dilutions of  $\text{Co}_3\text{O}_4$ -NPs and doxorubicin hydrochloride (Control drug) were made in water through sonication at room temperature for 10–15 min with 40 W to minimize agglomeration of NPs prior to cell viability assay. The suspension of  $\text{Co}_3\text{O}_4$ -NPs and doxorubicin hydrochloride were used for the treatment of cell culture with serial concentrations 0, 5, 10, 20, 40, 80, 160, 320 and 640  $\mu\text{g}/\text{mL}$ .

### MTT assay

MTT (dimethyl thiazolyltetrazolium bromide) assay was carried out to assess the viability of cancerous cell HT29 and SW620 as described by Mossman [27]. The MTT assay measures the enzymatic rate of mitochondrial succinate by quantifying ability of viable cells to reduce MTT into blue color formazan product. In brief, approximate  $1 \times 10^4$  cells/well were seeded in 96-well plates and exposed to  $\text{Co}_3\text{O}_4$ -NPs and doxorubicin hydrochloride with serial concentrations of 0, 5, 10, 20, 40, 80, 160, 320 and 640  $\mu\text{g}/\text{mL}$  for 24 h. Control cells (untreated) received 0  $\mu\text{g}/\text{mL}$   $\text{Co}_3\text{O}_4$ -NPs/doxorubicin hydrochloride + 200  $\mu\text{L}$  of culture medium containing 10 % dimethylsulfoxide in each experiment. At the completion of exposure, the culture medium was removed from each well to minimize the interference of  $\text{Co}_3\text{O}_4$ -NPs and changed with

freshly prepared MTT solution (with 0.5  $\text{mg}/\text{mL}$  concentration) in an amount equal to 10 % of initial culture volume and incubated for 2–3 h at 37 °C until a purple-colored formazan product developed. The resulting product of formazan was dissolved in acidified isopropyl alcohol. Moreover, the remaining NPs present in the solution were settled down through the centrifugation of 96-well plate at 2500 rpm for 5 min. Finally, a 100  $\mu\text{l}$  volume of each supernatant were transferred to corresponding wells of fresh 96-well plate and absorbance was measured at 590 nm using a microplate reader. The experiments were conducted in form of triplicates. The average of triplicates was employed to determine the  $\text{IC}_{50}$  value and survival fraction of the viable cells.

Optical density value was subjected to calculate the percentage of viability using the following formula:

% of cell viability

$$= \frac{1/4 \text{ OD value of experimental sample } \text{Co}_3\text{O}_4\text{-NPs} \times 100}{\text{OD value of experimental control untreated}}$$

Moreover, alteration in expression of anti-apoptotic markers such as Bcl2 and BclxL was assessed using western blot assay along with control  $\beta$ -actin.

### Estimation of expression of anti-apoptotic markers

Western blot assay was performed to estimate the alteration in expression of anti-apoptotic markers of apoptotic pathway such as Bcl2 and BclxL using human colorectal cancer cell HT29. Initially, cells were grown in DMEM (Invitrogen) supplemented with 10 % fetal bovine serum, 100 U/mL penicillin, 100  $\mu\text{g}/\text{mL}$  streptomycin and 2  $\text{mmol}/\text{l}$  L-glutamine at 37 °C under high humidity and 5 %  $\text{CO}_2$ . The HT29 cells were exposed with 0.5  $\text{mg}/\text{mL}$  concentration of  $\text{Co}_3\text{O}_4$ -NPs for 24 h. Whole cell lysates were prepared as described in our one of previous study [28]. The cell lysates were used for measuring of expression levels of soluble proteins content through SDS-PAGE immunoblotting. The membrane was then probed with Bcl2, BclxL (Santa Cruz Biotechnology) and  $\beta$ -actin (Sigma) antibodies to determine the alteration in expression of proteins. The reactivity of antibody against Bcl2, anti-BclxL was observed with horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (GE healthcare).

### Analysis of antibacterial potential of $\text{Co}_3\text{O}_4$ -NPs

The antibacterial potential of synthesized  $\text{Co}_3\text{O}_4$ -NPs was analyzed initially by disk diffusion method using following Gram-negative and -positive strains of bacteria.

## Gram-negative and -positive bacterial strains

Total nine bacterial strains, including five Gram-negative bacteria (*Staphylococcus aureus* ATCC-29213, *Pseudomonas aeruginosa* ATCC-27853, *Shigella sonnei* ATCC-11060, *Salmonella typhimurium* ATCC-13311, and *Proteus vulgaris* ATCC-6380) and four Gram-positive bacteria (*Escherichia coli* ATCC-35218, *Escherichia coli* ATCC-25922, *Enterococcus faecalis* ATCC-29212, and *Bacillus subtilis* NCTC-10400) were used for the analysis of antibacterial potential of  $\text{Co}_3\text{O}_4$ -NPs.

Bacteria were sub-cultured from pure cultures of different strain of bacteria on Mueller–Hinton Broth for overnight at 37 °C. The turbidity of bacterial culture was adjusted to 0.5 McFarland Standard. Each bacterial strain was swabbed uniformly onto separate agar plates using sterile cotton swabs in sterile condition. The sterile paper disks were putted on the agar plates, and 10  $\mu\text{L}$  of 250  $\mu\text{g}/\text{mL}$  (w/v) concentration of  $\text{Co}_3\text{O}_4$ -NPs were applied to the disks. The strips of control drug were used as control drugs. The MIC of  $\text{Co}_3\text{O}_4$ -NPs and control drugs was analyzed as per standard CLSI protocol (Clinical Laboratory Science Institute) [29].

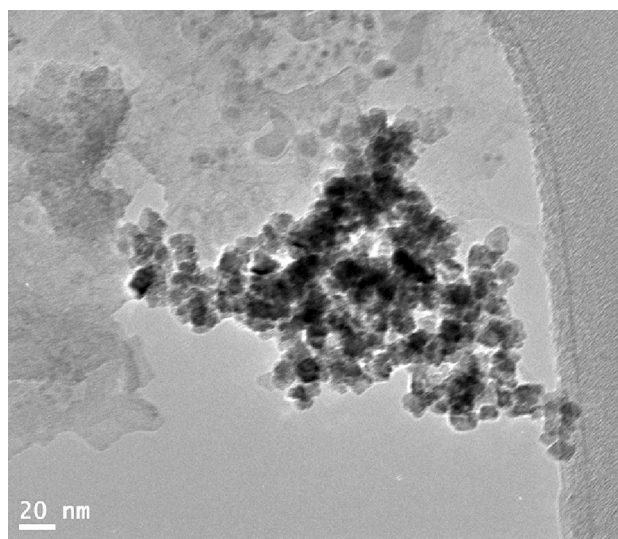
## Control drugs

The strips of Ampicillin and Cefotaxime were obtained from bioMérieux and Liofilchem. The standard MIC test strips of Ampicillin (bioMérieux) and Cefotaxime (Liofilchem) were used as control drug in the experiments to measure the potential of standards against nine bacterial strains. All the treated strains of bacteria with  $\text{Co}_3\text{O}_4$ -NPs and control drugs were incubated at 37 °C for 18 h. The tests were performed in form of triplicate. The zone of inhibition was measured and compared with standards, which appeared as a clear area around the disks and strips.

## Results and discussion

### Synthesis and characterization of $\text{Co}_3\text{O}_4$ -NPs

X-ray powder diffraction pattern of the urea-based thermally decomposed  $\text{Co}_3\text{O}_4$ -NPs is illustrated in Fig. S1 (Supporting information). The results of XRD pattern indicate that the  $\text{Co}_3\text{O}_4$ -NPs are well crystallized and pattern is in good agreement with the reported literature data [30]. The principle reflection peaks of  $\text{Co}_3\text{O}_4$  in diffraction pattern are measured, which correspond to the (220) and (311) planes. These reflection peaks can be indexed to the pure cubic fluorite structure of  $\text{Co}_3\text{O}_4$ . The intensities and positions of the diffraction plane are perfectly similar to the JCPDS card and no differences between them and reported



**Fig. 1** Transmission electron micrograph (FE-TEM) recorded from a drop-coated film of the colloidal ethanol solution of cobalt oxide nanoparticles ( $\text{Co}_3\text{O}_4$ -NPs)

data. The average crystallite size of  $\text{Co}_3\text{O}_4$ -NP was also found to be 36 nm.

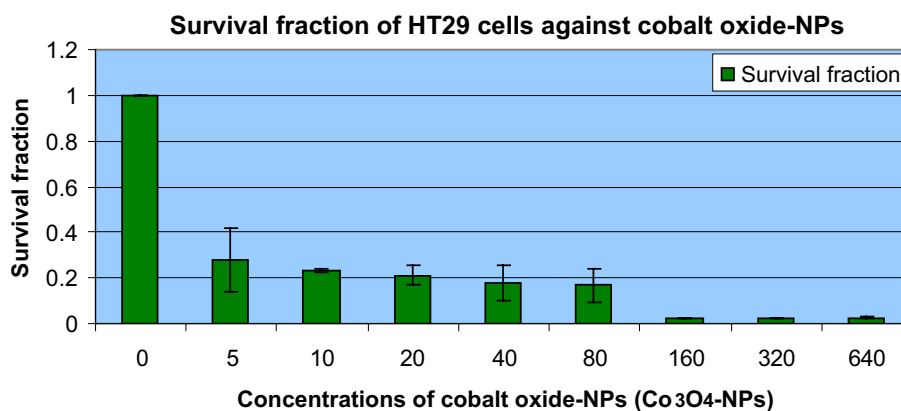
FETEM microscopy was used to examine the size and shape of the as-prepared cobalt oxide nanoparticles. Figure 1 illustrates the typical TEM microscopy image of  $\text{Co}_3\text{O}_4$ -NP. The average diameter of  $\text{Co}_3\text{O}_4$ -NP was determined by measuring over 100 particles in random fields of TEM view. Figure 1 shows that the  $\text{Co}_3\text{O}_4$ -NPs are non-spherical, aggregated, narrow distributed and irregular in shape with an average TEM diameter of about 20–25 nm which is supporting the XRD data.

### Cell viability assays

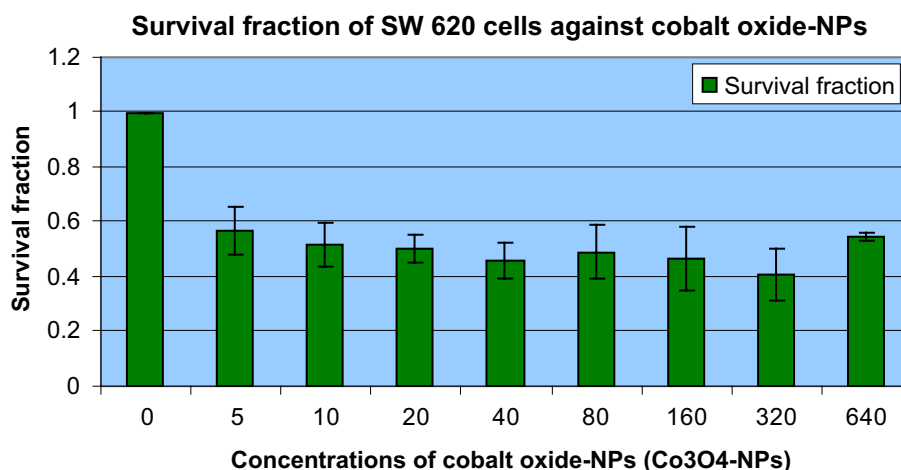
In vitro cytotoxic potential of  $\text{Co}_3\text{O}_4$ -NPs and doxorubicin hydrochloride was screened against HT29 and SW620 human colorectal cancer cell line and viability of tumor cells were confirmed using MTT assay. When cells were treated with various concentrations (0, 5, 10, 20, 40, 80, 160, 320 and 640  $\mu\text{g}/\text{mL}$ ) of  $\text{Co}_3\text{O}_4$ -NPs, for 24 h to calculating the value of  $\text{IC}_{50}$ , there was observed a significant decrease in cell viability compared to untreated cells as assumed the viability to be 1 (i.e., 100 %). Among HT29 and SW220, the percentage of cell viability was found to be less with HT29 cells. The results were showed that  $\text{Co}_3\text{O}_4$ -NPs induced significant potential cytotoxic response (Figs. 2, 3). On treatment of the HT29 and SW620 cells with  $\text{Co}_3\text{O}_4$ -NPs for 24 h at 37 °C in with a concentration of 5  $\mu\text{g}/\text{mL}$ , the outcome of the experiments shows slight change in cell viability. The cells (HT29 and SW620) treated with



**Fig. 2** Evaluation of HT29 Cell viability in terms of survival fraction after 24 h of exposure of different concentrations of  $\text{Co}_3\text{O}_4$  nanoparticles by MTT assay. The result showed the value of  $\text{IC}_{50}$  2.26  $\mu\text{g}/\text{mL}$ . Error bars represent standard deviation ( $n = 3$ )



**Fig. 3** Evaluation of SW620 cell viability in terms of survival fraction after 24 h of exposure of different concentrations of  $\text{Co}_3\text{O}_4$  nanoparticles by MTT assay. The result showed the value of  $\text{IC}_{50}$  394.5  $\mu\text{g}/\text{mL}$ . Error bars represent standard deviation ( $n = 3$ )



increasing concentrations (0, 5, 10, 20, 40, 80, 160, 320 and 640  $\text{mg}/\text{mL}$ ) of  $\text{Co}_3\text{O}_4$ -NPs for 24 h show a noticeable dose-dependent reduction in cell viability. The results of MTT assay demonstrated that  $\text{Co}_3\text{O}_4$ -NPs have a profound effect on a human colorectal cancer cells HT29 and SW620 with  $\text{IC}_{50}$  (inhibition of 50 % viable cells) value at 2.26 and 394.5  $\mu\text{g}/\text{mL}$ , respectively. Similarly, the outcome of MTT assay with different concentrations of doxorubicin showed that control drug doxorubicin also has a significant effect on a human colorectal cancer cells HT29 and SW620 with 113 and 151  $\mu\text{g}/\text{mL}$  values of  $\text{IC}_{50}$  (inhibition of 50 % viable cells), respectively. However, no significant effects were observed on normal cell MCF-10A (non-cancerous epithelial cell line) with treatment of different serial concentration of doxorubicin. The synthesized  $\text{Co}_3\text{O}_4$ -NPs did not exhibit significant decrease in cell mass against to normal cell line MCF-10A (non-cancerous epithelial cell line) with highest concentration (data not show). The enzyme mitochondrial dehydrogenase plays a crucial role in MTT assay which is used to measuring the cytotoxicity potential

in cell viability assay. The mitochondrial dehydrogenase enzyme cleaves the yellowish solution of tetrazolium MTT to purple color formazan crystals which is then dissolved through DMSO [27, 31]. Therefore, this mitochondrial enzyme which is present in metabolically active cells reduces MTT which indirectly indicates the percentage of viable cells [31].

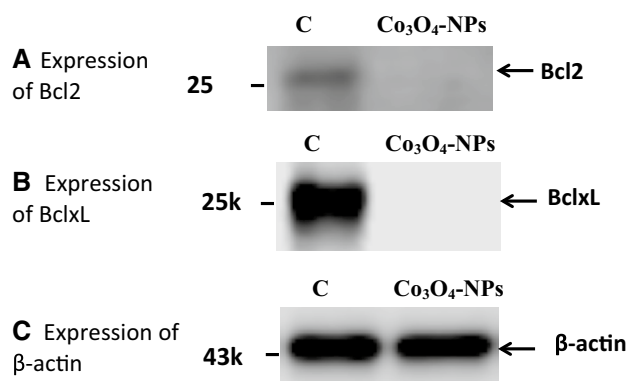
The result revealed the non-toxic nature and best biocompatibility of our synthesized  $\text{Co}_3\text{O}_4$ -NPs in vitro experiments against normal cell MCF-10A and toxic nature against cancerous cells. The outcomes of our study are in conformity with the modern fact that the mainly employed biocompatible material for the preparation of nanoparticles is the cobalt oxide. Interestingly,  $\text{Co}_3\text{O}_4$ -NPs have not exerted significant toxic effect as >80 % viability of normal cells MCF-10A at the highest concentration, whereas HT29 and SW620 cancerous cells show the profound cytotoxic effect. Therefore, it is suggested that the severe cytotoxicity mostly is initiated from the cellular internalization of  $\text{Co}_3\text{O}_4$ -NPs instead of physical injure on the cell membrane. Various studies

have confirmed that nanoparticles may enter into the cytoplasm, and nucleus of the host cells through different routes and strategies [32–35]. For instance, the various reports showed that the gold nanoparticles (GNPs) of 3–10 nm ranges entered into the nucleus of the host cells, whereas 25–50 nm GNPs accrued around the nucleus. [35, 36] The 3 nm size GNPs revealed maximum cytotoxicity while most of the large size GNPs showing less cytotoxic effect into the HEP-2 cells using MTT assay [36].

#### Estimation of expression of anti-apoptotic markers

Although the outcome of MTT assay indicated the significant toxic effect of  $\text{Co}_3\text{O}_4$ -NPs against HT29 cells, mechanistic approach is needed for the confirmation of these results. Furthermore, western blot assay with HT29 cells was used to determine both the mRNA and protein levels of Bcl2 and BclxL genes involved in apoptotic pathways. Therefore, the cytotoxic effect of  $\text{Co}_3\text{O}_4$ -NPs was studied through western blot technique to measure the expression levels of anti-apoptotic protein (Bcl2 and BclxL). These are two members of the Bcl-2 family that play a prominent role in the regulation of apoptosis. Expressions level of selected genes was compared to the  $\beta$ -actin gene, which was mainly used as an internal control due to housekeeping gene. The HT29 cells were treated with  $\text{Co}_3\text{O}_4$ -NPs at a concentration of 500  $\mu\text{g}/\text{mL}$  for 24 h. The results showed that the  $\text{Co}_3\text{O}_4$ -NPs significantly decrease the expression level of anti-apoptotic Bcl2 and BclxL gene product with comparison to control  $\beta$ -actin (a loading control) and untreated cells (act as negative control) (Fig. 4a, b). Results of our study also illustrated that no significant alteration observed in the expression level of loading control  $\beta$ -Actin and untreated negative control (Fig. 4c).

Various studies show that downregulation of the expression of Bcl2 and BclxL significantly enhances the process of programmed cell death in apoptosis pathway [37, 38]. Alternately, the upregulation of expression of Bcl2 and BclxL inhibits or decreases the efficiency of apoptosis by controlling of activation of caspase proteases [38]. The results of this study were revealed that the  $\text{Co}_3\text{O}_4$ -NPs inhibited the cellular proliferation significantly through the downregulation of expression of anti-apoptotic Bcl2 and BclxL gene product. Moreover, the observed results show that the expression of bcl-2 (antiapoptotic protein) was significantly low and the expression of BclxL (pro-apoptotic protein) was significantly high in the cells exposed to  $\text{Co}_3\text{O}_4$ -NPs (Fig. 3). Higher expression level of bax/bcl-2 proteins ratio in cells treated with  $\text{Co}_3\text{O}_4$ -NPs suggests that these 2 proteins also play a significant role in the pathway of  $\text{Co}_3\text{O}_4$ -NPs induced apoptosis. Therefore, these results of western blot assay with anti-apoptotic markers Bcl2



**Fig. 4** Western blot assay for analysis of alteration in expression of anti-apoptotic gene Bcl-2, Bcl-xL and compared with untreated control (c) and housekeeping gene  $\beta$  actin in HT29 cells after 24 h of exposure of 500  $\mu\text{g}/\text{mL}$   $\text{Co}_3\text{O}_4$  nanoparticles. Cells were lysed in buffer and cellular proteins were separated by SDS-PAGE and subjected onto PVDF membranes. The PVDF membranes were probed with primary antibodies (Anti-Bcl-2, Anti-Bcl-xL and Anti- $\beta$ -actin) and then secondary antibodies. The images of immunoblots were illustrated the expression level of anti-apoptotic proteins. **a** Downregulation of expression of Bcl-2. **b** Downregulation of expression of Bcl-xL. **c** The unchanged expression level of  $\beta$ -actin as an internal control

and BclxL corroborate with our previous outcome of MTT assay.

#### Analysis of antibacterial potential of $\text{Co}_3\text{O}_4$ -NPs

The antibacterial activity of  $\text{Co}_3\text{O}_4$ -NPs against four Gram-positive and five Gram-negative strain of bacteria is illustrated in Fig. S2 (Supporting information). The control drugs show antibacterial activity against various strains with a high reduction of bacteria growth, whereas the  $\text{Co}_3\text{O}_4$ -NPs did not demonstrate any significant antibacterial activity in 250  $\mu\text{g}/\text{mL}$  with comparison to control drugs (Fig. S2). The results of antibacterial potential of  $\text{Co}_3\text{O}_4$ -NPs were indicated  $>1000$   $\mu\text{g}/\text{mL}$  value of MIC of synthesized  $\text{Co}_3\text{O}_4$ -NPs against various strains of bacteria. The results of MIC were summarized and compared with control drugs (Table 1).

It has been assessed that our intestine contains approximately 1000 bacterial species and 100-time more genes than in the genome of human [39, 40]. This hidden metabolic community exerts immense impact on host immune function, physiology, metabolism and nutrition. It is currently evident that our gut microbiome co-evolves with us [41] and alterations in these communities can have major effects for human health. In fact, it has been suggested that dysbiosis of our microbiota can be important with respect to development of pathological condition including malnutrition [42], obesity [43, 44], systematic diseases like diabetes [45] and chronic inflammatory diseases like Crohn's

**Table 1** The MIC of Co<sub>3</sub>O<sub>4</sub>-NPs and control drugs was determined as per CLSI (Clinical laboratory standards Institute) protocol

Bacterial strains	MIC of Co <sub>3</sub> O <sub>4</sub> -NPs	AM (control)	CTX (control)
<i>Escherichia coli</i> ATCC 35218	>10,000	>256	0.032
<i>Staphylococcus aureus</i> ATCC 29213	>10,000	>256	1.5
<i>Escherichia coli</i> ATCC 25922	>10,000	>256	0.032
<i>Pseudomonas aeruginosa</i> ATCC 27853	>10,000	>256	24
<i>Enterococcus faecalis</i> ATCC 29212	>10,000	>256	1.5
<i>Bacillus subtilis</i> NCTC 10400	>10,000	>256	256
<i>Shigella sonnei</i> ATCC 11060	>10,000	256	0.016
<i>Salmonella typhimurium</i> ATCC 13311	>10,000	0.75	0.016
<i>Proteus vulgaris</i> ATCC 6380	>10,000	>256	24

The MIC of control was determined through commercial E-test strips. All values are in µg/mL; AM ampicillin, CTX cefotaxime

disease (CD), encompassing ulcerative colitis (UC) and inflammatory bowel disease (IBD) [46]. On the basis of non-significant antibacterial potential of Co<sub>3</sub>O<sub>4</sub>-NPs, it can be assumed that the synthesized Co<sub>3</sub>O<sub>4</sub>-NPs will exert toxic effect on cancerous cells where as the human microbiome including varieties of bacteria will be almost unaltered.

## Conclusion

In this present study, we observed that as-prepared Co<sub>3</sub>O<sub>4</sub>-NPs have excellent stability in aqueous medium with reasonable best hydrodynamic size. In vitro cell viability tests using an MTT assay revealed that synthesized Co<sub>3</sub>O<sub>4</sub>-NPs have great anti-cancer potential against cancerous cell lines; rather than they have no significant toxic effect on normal cells MCF-10A. Furthermore, western blot analysis has confirmed that Co<sub>3</sub>O<sub>4</sub>-NPs are targeting more effectively the cancerous cells through downregulation of anti-apoptotic protein Bcl2 and BclxL. Due to significant downregulation of expression of Bcl2 and BclxL, the synthesized Co<sub>3</sub>O<sub>4</sub>-NPs in our study may further be investigated for the use of novel anti-cancer drug development therapy. Results of our study suggested that further in vivo researches are required to identify their therapeutic index in cancer management and treatment. In vivo testing for anti-cancer potential of these Co<sub>3</sub>O<sub>4</sub>-NPs is in progress for the next-generation drug development.

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## Compliance with ethical standards

**Conflict of interest** The authors do not have any potential conflict of interest related to current work.

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