Evaluation cytogenotoxicity of cobalt oxide nanoparticle in mice liver


1Faculty of Applied Health Science technology, 6th October University Egypt.
2,3,4 Faculty of Science zoology Dept. Cairo University Egypt.
5,6 Faculty of Veterinary Medicine Benha University Egypt.
7 Biophysics department, Faculty of Science, Cairo University, Giza, Egypt.

*Corresponding author: Gehan. B. A Youssef, Faculty of Veterinary Medicine Benha University Egypt, Email: gihan.basiony@fvtm.bu.edu.eg

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ABSTRACT
This study was done on 4 groups of mice to evaluate the cytogenotoxicity of cobalt oxide nanoparticles by comet assay from liver tissue and bone marrow to detect DNA damage and the histopathological changes in the hepatic tissues. Significant elevation in %DNA, tail length, and tail moment in liver while in bone marrow the %DNA, tail length, and tail moment showed elevation in 5% cobalt oxide nanoparticles and decline in 10% and 20% of cobalt oxide nanoparticles. The liver tissues of the 5% cobalt nanoparticle group had areas of hepatocellular necrosis, focal aggregations of mononuclear inflammatory cells, and portal congestion, whereas the 10% cobalt oxide nanoparticle group had marked inflammatory cell infiltration, marked vascular congestion, and small focal areas of hepatocellular necrosis that were infiltrated by mononuclear inflammatory cells. The hepatic tissue parenchyma in the 20% cobalt oxide nanoparticles group had clogged blood arteries, and regions of hepatocellular necrosis with mononuclear inflammatory cell infiltration were often seen lesions in the liver. Some liver tissues, however, showed only little improvement. In conclusion the cobalt oxide nanoparticles were showed cytotoxicity and genotoxicity especially in concentration of 5% and 10%.

Keywords: Cobalt oxide nanoparticles, Cytogenotoxicity, Comet assay, Liver

INTRODUCTION
Cobalt is a magnetic mineral, and the cobalt nanoparticle has special Properties, including a large surface area that is associated with its small size and its catalytic and magnetic properties [1].Nano technological products have been used widely in fields ranging from medicine to industry because of their physicochemical properties. However, the human and environmental concern are gradually increased [2]. It is well known that nanoparticles be absorbed through the skin, ingested, and inhaled during occupational and/or environmental applications [3]. Based on the emerging biological roles of cobalt, cobalt oxide nanoparticles (including CoO NPs, Co2O3 NPs and Co3O4 NPs) have been applied in different fields because of their special physical and chemical properties [4,5] Nanotechnology is the creation, examination, and prospective use of different materials with special qualities at the nanoscale, defined as being smaller than 100 nm in at least one dimension [6].
It has been widely utilized in healthcare for the diagnosis and treatment of illnesses, for medication delivery, and for the development of innovative drug formulations [7]. A transition metal that is good for human health is cobalt [8]. It is a part of vitamin B12, which promotes the development of red blood cells and helps cure anaemia [9].

The two main kinds of genotoxicity caused by nanomaterials are primary genotoxicity, in which NPs directly target the cells, and secondary genotoxicity, in which genotoxicity results from off-target events affecting other cell types. Moreover, in primary genotoxicity, induction particles can interact with genetic material directly or through inducing DNA damages brought on by oxidative stress. Apart from these, particles can also influence processes such as cell cycle progression, DNA repair, replication, and chromosomal segregation indirectly by interfering with certain proteins [10].

Nanoparticles (NPs) are both harmful and beneficial to human health due to their quick absorption by cells. Recently, it was proposed that certain NPs could enter the cytoplasm by passing through the plasma membrane via a non-endocytotic mechanism [11].

Nevertheless, the primary problem preventing their application in the treatment and detection of illnesses is their toxicity for living things. Nowadays, toxicity-related issues and side effects are a common difficulty for researchers. In this regard, it is crucial to select an appropriate experimental paradigm for determining toxicity in vitro (using cell lines) and in vivo (using experimental animals). Depending on their physicochemical properties and method of manufacture, NPs can enter the body through the skin, inhalation, or digestion [12].

Depending on the kind of chemicals in NPs, the body can interact with them through the respiratory system, the digestive system, the skin, or the blood [13]. There are worries about the hazards that certain NPs, such ZnO and TiO2, which have the capacity to block UV rays and are widely employed in several health goods on the market, may pose to human health, public safety, and the environment when they are disseminated in the environment.

Primary investigations reveal that NPs may enter the human body in a variety of ways, accessing important organs via blood flow and causing tissue and cell damage [14]. Researchers have linked factors such particle form, size, dispersity, surface charge, and protein corona effects to the toxicity of NPs, despite the fact that the mechanism of action of NPs in this respect is not fully understood.

According to several studies, NPs increase oxidative stress and the expression of genes related to inflammation [15]. NPs may enter the human body by breathing, eating, or being injected, and they can then build up in various tissues and organs [16]. By severing the tight injunction in the blood-brain barrier (BBB) and attaching to cells that possess the CXCR6 chemokine receptor, NPs can even cross the blood-brain barrier to the brain [17]. The movement of the NPs through the membrane, their functionality, and their impact on cell metabolism are currently being investigated and discussed. In order to address the question of whether NPs have harmful and toxic effects on organs or if they are sufficiently safe, we attempt to describe a component of their performance here [18]. Only by fully comprehending the interconnections between all of the variables and mechanisms generating NP toxicity can safe, biocompatible NPs be developed that can be used to the detection and treatment of human illnesses.

Several biological reactions, such as adhesion, proliferation, and differentiation, are caused by the surface characteristics of nanoparticles. Since NPs produce ions that cause toxicity on the cell membrane surface, they interact physically and chemically with the cell membrane to cause oxidative stress, which may then be used to destroy cancer cells [19].

The toxicity of cobalt based nanoparticles, may be characterized either to the direct uptake of the NPs by cells or to the dissolution of the NPs leading to the increased level of Co+++ ions in the media, with subsequent effects on cells. There are a number of studies related to the potential toxicity of cobalt based nanoparticles. Cobalt oxide nanoparticles were found to exert an oxidative stress in cells [20], DNA damage [21,22], and inflammatory response [23], in
human mononuclear cells [24], and neutrophils [25].

CoO-NPs have been found to be genotoxic and carcinogenic both in vitro and in vivo [26,27]. CoO-NPs have been shown in in vitro experiments to promote the development of micromolecules, oxidative DNA damage, and double stranded DNA breaks in a variety of experimental cell lines [28,29]. CoO-NPs were administered to rats over a two-year period, and an in vivo investigation revealed that this resulted in benign lung tumours, bronchio-alveolar carcinoma, adenocarcinomas, and bronchio-alveolar adenomas [30]. CoO-NPs-induced genotoxicity is currently thought to have a plausible mechanism involving oxidative stress. Many research have used various experimental settings to show that CoO-NPs can produce reactive oxygen species (ROS) [31].

**MATERIAL AND METHODS**

*Preparation of cobalt oxide nanoparticles*

Co (II) nitrate (Co (NO3)2. 6H2O and Sodium carbonate Na2CO3, ACS reagent specified 98% and 99.5% respectively pure were purchased from Sigma-Aldrich and used without further purification. Co3O4 nanoparticles were synthesized using the optimized precipitation method as described in detail [32], where 0.1M (Co (NO3)2. 6H2O solution was added to 0.01M Na2CO3 solution at a flow rate 2.5 ml/min at 30°C under strong stirring rates. The reaction product, CoCO3 filtered and washed with distilled water and ethanol several times before drying at 80°C for 3h. CoCO3 as a precursor to the Co3O4 nanoparticles were heated at 400°C for 2 h to give pure Co3O4-NPs as shown by X-ray diffractometer (XRD).

The specification of the size and structure of the Co3O4 nanoparticles were carried out. X-ray (XRD) was used to identify the crystalline phase and to estimate the crystalline size. The XRD pattern were recorded with 20 in the range of 20 - 85° with type Malvern Pananalytical X-Pert Pro MPD, Cu-Kα: λ = 1.54 A.

Using a Zetasizer Nanoseries, dynamic light scattering (DLS) was used to calculate the polydispersity (% Pd) of the distribution and the mean diameter of the Co3O4 nanoparticles (Malvern Instruments, UK). The sample was tested three times using the unimodal model for size distribution and the calculation factors utilised were the refractive index and viscosity of pure water. The results are provided as a mean ±SD for the polydispersity and mean diameter of the Co3O4 nanoparticles. At 25 °C, the size distribution was observed as a function of volume.

**Animals**

Mice were obtained from National Cancer Institution (NCI) Animal house Unit. All mice have left in the animal house for one week under standard dark/light cycle to be acclimatized and supplied with standard diet pellets and water ad libitum. IACUC Protocol Number (CU I F 30 21).

**Genotoxicity assessment**

-Safety dose of Cobalt oxide nanoparticles 2000 mg/kg

-20 normal healthy male mice were randomly divided into four groups; five mice each as follow:

Group 1: Healthy negative control group in which mice were orally administered standard diet pellets and deionized dist. Water

Group 2: Healthy mice were orally administered Cobalt oxide nanoparticles at the dose level 5% of the safety dose daily for two weeks

Group 3: Healthy mice were orally administered Cobalt oxide nanoparticles at the dose level 10% of the safety dose daily for two weeks

Group 4: Healthy mice were orally administered Cobalt oxide nanoparticles at the dose level 20% of the safety dose daily for two weeks

**Collection of organs**

-Animals were anesthetized by using isoflurane (1-4%) and sacrificed by cervical dislocation after 24 hour of the last administration. Samples were collected from mice liver for histopathological analyses.
Detection of DNA damage using Comet assay
To assess the DNA damage level in liver and bone marrow of genotoxicity groups alkaline comet assay (Ph >13) was done according to the method described by [33 ].

Histological examination
Small pieces of the liver tissue from each group were collected and preserved in bounis solution for histological analysis according to [34 ].

Statistical analysis
At least three independent runs of each experiment were completed. Unless otherwise noted, the data shown here are mean SE. Data were analysed using GraphPad Prism's one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests to compare the control and treatment groups (V0.5.01). P-values under 0.05 were used to determine statistical significance for values.

RESULTS
The experimental X-ray powder diffraction (XRD) pattern of the synthesized Co3O4 nanoparticles is shown in Figure 1 to identify crystalline phases and to estimate the crystalline sizes.

FIGURE 1: XRD Pattern of the Co3O4 nanoparticles synthesized using the optimized precipitation method.

The DLS size distribution of Co3O4 nanoparticles, as a function of volume at 25 °C, was unimodal and relatively narrow Figure 2.

FIGURE 2: Size distribution measured by (DLS) of the of the Co3O4 nanoparticles synthesized using the optimized precipitation method.
Comet assay
In liver tissue %DNA damage was (Table.1) significantly higher 15.91±1.55 in cobalt oxide nanoparticle 20% than the control 5.24±0.32, while the tail length was significantly higher in 30.55±3.13 cobalt oxide nanoparticle 10% and the tail moment was significantly higher 8.89±3.14 in cobalt oxide nanoparticle 20% than the control (Figure.3 and 3a).

**FIGURE 3**: photomicrograph of liver comet assay showing; normal nucleus (A), 5% cobalt oxide nanoparticles (B), 10% cobalt oxide nanoparticles (C) and 20% cobalt oxide nanoparticles (D).

**TABLE 1**: Effect of different concentration of cobalt oxide nanoparticles on DNA damage level in liver tissue

<table>
<thead>
<tr>
<th>compounds</th>
<th>Liver</th>
<th>Control Mean ± SD</th>
<th>5% Mean ± SD</th>
<th>10% Mean ± SD</th>
<th>20% Mean ± SD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td></td>
<td>5.24±0.32</td>
<td>10.11±0.4a</td>
<td>12.16±1.7a</td>
<td>15.91±1.55abc</td>
<td>42.479</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DNA %</td>
<td></td>
<td>18.87±1.71</td>
<td>25.25±0.76a</td>
<td>30.55±3.13a</td>
<td>24.04±2.07a</td>
<td>15.468</td>
<td>.001</td>
</tr>
<tr>
<td>Moment</td>
<td></td>
<td>1.45±0.10</td>
<td>4.06±0.06</td>
<td>7.43±1.26ab</td>
<td>8.89±3.14ab</td>
<td>35.408</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A: Significant with control group at p < 0.05
B: Significant with 5% group at p < 0.05
C: Significant with 10% group at p < 0.05

**FIGURE 3A**: Effect of different concentration of cobalt oxide nanoparticles on DNA damage level in liver tissue
In bone marrow tissue, %DNA damage, tail length, and tail moment were (Table 2) higher in cobalt oxide nanoparticles 5% than the control 5.24±0.32, while the other concentration 10% and 20% of the cobalt oxide nanoparticle were lowered than that of the control group (Figure 4 and 4a).

![Photomicrograph of bone marrow comet assay](image)

**FIGURE 4:** Photomicrograph of bone marrow comet assay showing; normal nucleus (E), 5% cobalt oxide nanoparticles (F), 10% cobalt oxide nanoparticles (G) and 20% cobalt oxide nanoparticles (H).

**TABLE 2:** Effect of different concentration of cobalt oxide nanoparticles on DNA damage level in bone marrow tissue

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Length</td>
<td>11.64±0.48</td>
</tr>
<tr>
<td>DNA</td>
<td>33.2±0.37</td>
</tr>
<tr>
<td>Moment</td>
<td>6.5±0.34</td>
</tr>
</tbody>
</table>

A: Significant with control group at p < 0.05  
B: Significant with 5% group at p < 0.05  
C: Significant with 10% group at p < 0.05

![Bar chart showing DNA damage level](image)

**FIGURE 4A:** Effect of different concentration of cobalt oxide nanoparticles on DNA damage level in bone marrow tissue.
**Histopathology results**

The hepatic tissues of the control group (Fig. 1 and 2) consisted of hepatic cords around central vein. The hepatic cords consisted of hepatocytes with basophilic nuclei and acidophilic cytoplasm. The liver tissues were revealed areas of hepatocellular necrosis with existence of focal aggregations of mononuclear inflammatory cells and portal congestion in 5% cobalt group (Fig. 3 and 4). The 10% cobalt group showed substantial vascular congestion in the portal regions, along with tiny localized foci of hepatocellular necrosis that were invaded by mononuclear inflammatory cells. (Fig. 5 and 6). The hepatic tissue parenchyma in the 20% cobalt group displayed clogged blood arteries, and regions of hepatocellular necrosis with infiltration of mononuclear inflammatory cells were often seen lesions in the liver. While other hepatic tissues showed moderate improvement (Fig. 7 and 8).

**FIG. (1)** Photomicrograph of liver, Control group showing normal hepatic parenchyma (H&E).
FIG. (2) Photomicrograph of liver, Control group higher magnification showing normal hepatic parenchyma (H&E).

FIG. (3) Photomicrograph of liver, Cobalt 5% group showing small focal aggregation of mononuclear inflammatory cells (arrow) (H&E).

FIG. (4) Photomicrograph of liver, Cobalt 5% group showing small focal aggregation of mononuclear inflammatory cells (arrow) (H&E).

FIG. (5) Photomicrograph of liver, Cobalt 10% group showing an area of hepatocellular necrosis (arrow) with mononuclear inflammatory cells infiltration as well as portal infiltration with inflammatory cells (arrow) (H&E).

FIG. (6) Photomicrograph of liver, Cobalt 10% group higher magnification showing an area of hepatocellular necrosis (arrow) with mononuclear inflammatory cells infiltration (H&E).

FIG. (7) Photomicrograph of liver, Cobalt 20% group showing an area of hepatocellular necrosis (arrow) with mononuclear inflammatory cells infiltration (H&E).

FIG. (8) Photomicrograph of liver, Cobalt 20% group higher magnification showing an area of hepatocellular necrosis (arrow) with mononuclear inflammatory cells infiltration (H&E).

DISCUSSION

The liver tissues were showed areas of hepatocellular necrosis with existence of focal aggregations of mononuclear inflammatory cells and portal congestion in 5% cobalt oxide nanoparticles while the portal areas exhibited marked inflammatory cells infiltration in 10% cobalt oxide nanoparticles group with marked vascular congestion with presence of small focal areas of hepatocellular necrosis that were infiltrated by mononuclear inflammatory cells. In 20% cobalt oxide nanoparticle group the hepatic tissue parenchyma showed congested blood vessels and areas of hepatocellular necrosis with mononuclear inflammatory cells infiltration were the frequently detected lesions in liver. While other hepatic tissues showed moderate improvement. Reports indicating Co3O4 nanoparticles drastically decreased cell viability and caused apoptosis, oxidative stress, caspase activity, and the production of pro-inflammatory cytokine genes in vitro confirm our findings. After exposure, they suggested that the negative impacts of Co3O4 and TiO2 nanoparticles could increase cardiovascular risk in people. Similar findings suggested that the caspases were important in the beginning and completion of apoptosis, resulting in cellular DNA damage in human cells caused by Co3O4 nanoparticles. Co3O4 nanoparticles were discovered to significantly cause cell death by the generation of ROS, which then caused tumour necrosis factor-α by activating pro-apoptotic proteins in human leukaemia cells (p38-MAPK, caspase-8, and caspase-3). Nevertheless, Co3O4 nanoparticle toxicity in the liver, gastrointestinal tract, and nervous system was observed to be extremely minimal. As a consequence, the findings suggest that Co3O4 nanoparticles may be employed in industrial, commercial, and nanomedical applications without risk if the exposure dosage is managed at the exposure pathways.

Time and dose both had an impact on the cytotoxic effects of Co2+ and Co-NPs, with Co-NPs having a higher cytotoxicity than Co2+. Also, after 24 hours of exposure, Co-NPs caused a substantial (p 0.05) decrease in cell viability along with a simultaneous rise in lactate dehydrogenase release, reactive oxygen species production, IL-8 mRNA expression, Bax/Bcl-2 mRNA expression, and DNA damage. NiO-NPs or Co3O4-NPs administered orally once each induced DNA strand breaks, apoptosis damage, and excessive ROS formation that greatly elevated MDA levels while markedly lowering Zn, GSH, and SOD activity in the brain, liver, and kidney tissues. Second, the effects of NiO-NPs or Co3O4-NP administration on all parameters were time- and dose-dependent in the tissues of the rats. Lastly, the genotoxicity and oxidative stress caused by each of the NPs were significantly reduced when NiO-NPs and Co3O4-NPs were administered combined.

Our results showed that significant elevation in %DNA, tail length, and tail moment in liver while in bone marrow the %DNA, tail length, and...
CONFLICT OF INTEREST
The authors declare no conflict of interest

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