



Evaluation of some biological, biochemical, and hematological aspects in male albino rats after acute exposure to the nano-structured oxides of nickel and cobalt

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Abstract

Nanomaterial applications are a fast-developing field. In spite of their powerful advantages, many open questions regarding how these small-sized chemicals may influence the environment and human health. However, scarce reports are available on the potential hazards of combined nanoparticles, taken into consideration that nickel oxide (NiO) and cobalt (II, III) oxide (Co₃O₄) nanoparticles (NPs) are already used together in many applications. Hence, the present work was designed to study the probable changes in some biological, hematological, and serum biochemical variables throughout 2 weeks following an oral administration of 0.5 g and 1.0 g of NiO-NPs or/ and Co₃O₄-NPs per kilogram body weight of rats. As compared with the controls, the exposure to NiO-NPs or Co₃O₄-NPs solely caused significant elevations in the relative weights of brain (RBW), kidney (RKW) and liver (RLW), water consumption (WC), red blood cells (RBCs) count, hemoglobin (Hb) content, packed cell volume (PCV), and serum levels of low-density lipoprotein cholesterol (LDL-C), glucose, creatinine, urea, and uric acid as well as serum activities of aspartate and alanine aminotransferases (ASAT and ALAT). In addition, remarkable declines in the total body weight (TBW), feed consumption (FC), white blood cells (WBCs) count, serum levels of total protein (TP), albumin, albumin/globulin ratio, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were caused by administration of NiO-NPs or Co₃O₄-NPs, separately. On contrary, the co-administration of NiO-NPs and Co₃O₄-NPs together caused less noticeable changes in most of studied variables as compared with those administered NiO-NPs or Co₃O₄-NPs, individually. In conclusion, the exposure to a combination of NiO-NPs and Co₃O₄-NPs suppressed the adverse effects of the individual NPs on the studied variables.

Keywords Nanoparticles · Cobalt oxide · Nickel oxide · Biological variables · Metabolism · Hematology

Introduction

Nanomaterials are structures with at least one of their dimensions have an average diameter of less than 100 nm (Magaye et al. 2012). Currently, the nanoparticles (NPs) are involved in nearly all the fields of our lives including the cosmetics, electronics, food, drug delivery, and medicine (Raj et al. 2012). Regrettably, the excessive utilization of the NPs may lead to adverse impacts on human health as well as the total environ-

ment (Hristozov and Malsch 2009). Such adverse effects can be linked to the unique properties of the NPs with respect to the shape, size, and surface area (Auffan et al. 2009; Maurer-Jones et al. 2013). Recently, there is a great interest towards the metal oxide nanoparticles (MO-NPs) due to their incomparable properties (Ates et al. 2016). The MO-NPs of transition metals including iron oxide (Fe₂O₃), nickel oxide (NiO), zinc oxide (ZnO), copper oxide (CuO), and cobalt oxides (CoO and Co₃O₄) are widely used in many applications.

NiO-NPs are extensively used in optical and electronic devices like batteries and fuel cells (Siddiqui et al. 2012) as well as in urea and glucose sensors (Mishra et al. 2018; Parsaee 2018). NiO-NPs induced oxidative stress-mediated injury in human lung carcinoma cells (Horie et al. 2011). Human breast cancer and airway epithelial cells viability was suppressed by NiO-NPs, in dose-dependent manner (Siddiqui et al. 2012). Moreover, after 72 h of a single intratracheal instillation with

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NiO-NPs, severe injury was developed in lung tissue of rats (Horie et al. 2011). Furthermore, hepatotoxicity was induced in male Wistar rats after 6 weeks of repeated exposure to 0.015, 0.06, and 0.24 mg per kg body weight of NiO-NPs via intratracheal instillation (Liu et al. 2017).

Nano-structured Co_3O_4 is a crucial MO-NP that has interesting catalytic and magnetic properties (Farhadi et al. 2016). Co_3O_4 -NPs are widely used in some practicable applications such as photocatalysts (Warang et al. 2012), batteries (Du et al. 2007) and gas sensors (Wu et al. 2008). An exposure to Co_3O_4 -NPs for 4 months has induced cytotoxicity in human-derived keratinocytes (Mauro et al. 2015). After 24 h of incubating human lymphocytes with 10 $\mu\text{g}/\text{mL}$ of nano-sized cobalt oxide, significant toxicity was observed (Chattopadhyay et al. 2015). Co_3O_4 -NPs caused a dose- and time-dependent impairment of cellular viability of endothelial-like cells (ECV-304) and hepatoma cells (HepG2) (Papis et al. 2009). In addition, the liver, spleen, and kidney of Swiss mice were adversely affected after 15 days of subcutaneous injection with 200–1000 $\mu\text{g}/\text{kg}$ body weight of cobalt oxide nanoparticles (Chattopadhyay et al. 2015).

In nature, chemicals are not found individually in the environment. Metals such as nickel and cobalt can be found together in plants (Kayode and Yakubu, 2017) and as alloys in soil and dust (Suh et al. 2019). Moreover, nickel and cobalt are used together in production of mixed nanosheets to accelerate their electrochemical activation (Schneiderová et al. 2017). Unfortunately, most of the studies on NPs were conducted to evaluate the toxic effects of the individual NPs rather than their combined effects. Thus, the present study aimed at assessing the combined effect of NiO-NPs and Co_3O_4 -NPs on the some biological, hematological, and biochemical aspects of the male albino rats.

Materials and methods

Nanoparticles

Nanoparticles were obtained from Sigma Aldrich (Ward Hill, MA, USA). According to the manufacturer data sheet, the mean particle diameters of NiO-NPs and Co_3O_4 -NPs were less than 50 nm. The black NiO nanopowder was 99.8% pure with density of 6.67 g/mL at 25 °C. However, Co_3O_4 -NPs (gray powder), with 99.5% purity and density of 6.11 g/mL at 25 °C, were used. Characterization of NPs via transmission electron microscope (TEM), X-ray diffraction (XRD), and dynamic light scattering (DLS) was performed in a separate study (Ali 2018). The average particle diameter, hydrodynamic size, poly-dispersion index, and zeta potential were 16.90 nm, 91.54 nm, 0.394, and + 42.1 mV, for the NiO-NPs, and 20.68 nm, 92.03 nm, 0.235, and + 41.5 mV for the Co_3O_4 -NPs, respectively.

Experimental model

The used experimental animal was the adult male Wistar rats, *Rattus norvegicus*, with a starting body weight of ca. 120 ± 10 g. The rats were obtained from the animal facility of the National Research Centre (NRC), Dokki, Giza, Egypt. The animals were acclimated for 1 week, prior to the experiments, in a clean polyethylene cages (five rats per cage) in the animal house of Zoology Department, Faculty of Science, Cairo University, Egypt. The animals were kept at a room temperature (22–25 °C) and were subjected to 12-/12-h light-dark cycle. The rats were given a free access to water and normal rodent chew. The cages were cleaned to get rid of feces and debris day after day.

Experimental design

The rats were randomly divided into seven equal groups as shown in Fig. 1.

Dose preparation

Stock solutions of the nano-sized NiO and Co_3O_4 were prepared by suspending these NPs into 0.5% CMC (Srivastav et al. 2016). The suspensions were ultra-sonicated for 15 min using ultrasonic homogenizer (BioLogics, Inc., Manassas, VA, USA), immediately before the administration. Doses of 0.5 and 1.0 g/kg of NiO-NPs and/or Co_3O_4 -NPs were administered once orally as “effective acute doses” as the median lethal of the tested NPs was estimated to be > 2.0 g/kg (Ali 2018). The rats were dosed according to their total body weights. The volume of each dose was adjusted to be 2 mL/100 g of body weight.

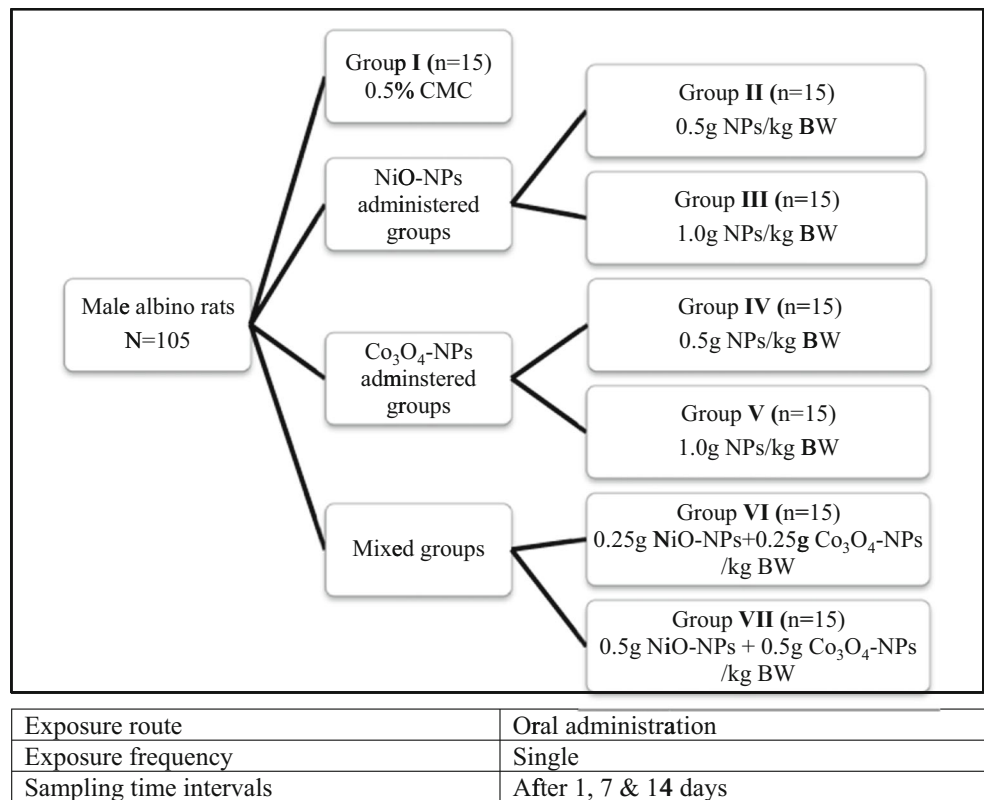
Body weight and consumption rates of food and water

The total body weight (TBW) of the experimental rats was recorded at 0, 1, 7, and 14 days. Each experimental group was divided into three cages with five rats per cage. The rates of feed consumption (FC) and water consumption (WC) for each cage were recorded daily to calculate the average daily consumption per rat.

Sampling

Five rats were randomly collected from each group at each time interval (1, 7, and 14 days). Rats were euthanized by an over dose of Na-pentobarbital, then the jugular vein was cut with sharp scalpel. The drained blood was collected into two sterile tubes. The first tube was EDTA-coated and used for further hematological analysis. The other portion was drained into dry test tubes for serum separation by centrifugation at 3000 rpm for 15 min. The serum was then stored in deepfreeze at -20 °C. Rats were

Fig. 1 A brief study design. BW body weight, CMC carboxymethyl cellulose, *N* total sample size, *n* group size



immediately dissected to remove the desired tissues. The brain, kidney, and liver were weighed and relative (to body weight) organ weights were calculated and expressed as RBW, RKW, and RLW, respectively (Loveless et al. 2008).

Hematological assay

The counts of red and white blood cells (RBCs and WBCs), hemoglobin (Hb) content, and packed cell volume (PCV) were analyzed using automated blood analyzer (HA-Vet CLINDIAG, Delhi, India).

Biochemical assay

In the serum of all the experimental groups, the levels of total cholesterol (TC), triglycerides (TG), low- and high-density lipoprotein cholesterols (LDL-C and HDL-C), total protein (TP), albumin, globulin, albumin/globulin ratio, glucose, urea, uric acid, and creatinine as well as the activities of aspartate and alanine aminotransferase (ASAT and ALAT) were measured colorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt).

Statistical analysis

Statistical analysis was executed using Statistical Package of the Social Sciences; SPSS version 23. Least significant difference (LSD) test was executed to study statistically significant differences in variables between the experimental times.

Duncan’s test were utilized to examine the homogeneity among all the experimental groups. Data was displayed as mean ± standard error of mean.

Results

Effect of nanoparticles on biological variables

Table 1 shows the TBW, RBW, RKW, RLW, FC, and WC of all the studied groups, throughout the experiments. At the first day, all the experimental rats administered NiO-NPs or/and Co₃O₄-NPs showed significant declines in the TBW, as compared with their starting body weights, at zero day. However, TBW of groups VI and VII were markedly greater than those administered NiO-NPs or Co₃O₄-NPs, individually, at the first day. By increasing the experimental time, TBW of all groups was increased significantly. On the fourteenth day, TBW of all groups was similar to the controls. At the first day, in rats administered NiO-NPs or Co₃O₄-NPs, relative weights of most organs were significantly increased by increasing the administered dose of NiO-NPs, whereas they markedly decreased by elevating the doses of Co₃O₄-NPs. By increasing the experimental time, most organs of rats administered NiO-NPs or Co₃O₄-NPs exhibited significant declines in their relative weights. In rats of groups VI and VII, all relative organ weights were similar to the controls, at any experimental period. On the first day, all the rats administered with NiO-NPs

Table 1 The total body weight (TBW), relative weights of the brain (RBW), liver (RLW) and kidney (RKW), feed consumption (FC), and water consumption (WC) of all the experimental groups, throughout the experiments

Parameter	Tim (day)	Experimental group						
		I	II	III	IV	V	VI	VII
TBW (g)	0	171.05 ± 0.47 ^a	170.55 ± 0.28 ^a	170.69 ± 0.10 ^a	172.44 ± 0.60 ^a	172.28 ± 0.88 ^a	172.31 ± 1.98 ^a	172.43 ± 0.73 ^a
	1	171.33 ± 0.30 ^c	153.02 ± 0.33 ^{af}	152.88 ± 0.67 ^{af}	154.40 ± 2.01 ^{af}	155.06 ± 0.94 ^{af}	159.49 ± 0.50 ^{bf}	158.28 ± 0.62 ^{bf}
	7	186.20 ± 1.36 ^{c*}	177.03 ± 0.86 ^{ab*}	170.92 ± 0.22 ^{a*}	178.82 ± 1.48 ^{b*}	177.40 ± 2.07 ^{ab*}	179.30 ± 3.18 ^{b*}	180.42 ± 3.67 ^{bc*}
	14	214.10 ± 1.28 ^{a*#}	213.45 ± 1.38 ^{a*#}	208.55 ± 0.43 ^{a*#}	214.92 ± 3.03 ^{a*#}	213.40 ± 7.14 ^{a*#}	211.07 ± 0.64 ^{a*#}	206.28 ± 7.16 ^{a*#}
RBW (g 100 g ⁻¹ BW)	1	0.86 ± 0.02 ^a	1.01 ± 0.01 ^c	1.06 ± 0.01 ^d	0.95 ± 0.01 ^b	0.90 ± 0.02 ^a	0.90 ± 0.01 ^a	0.89 ± 0.03 ^a
	7	0.87 ± 0.04 ^a	0.90 ± 0.01 ^{ab*}	0.95 ± 0.01 ^{b*}	0.92 ± 0.02 ^{ab}	0.86 ± 0.01 ^a	0.88 ± 0.02 ^{ab}	0.88 ± 0.03 ^{ab}
	14	0.87 ± 0.02 ^{ab}	0.84 ± 0.04 ^{a*}	0.94 ± 0.02 ^{b*}	0.89 ± 0.01 ^{ab}	0.84 ± 0.02 ^a	0.84 ± 0.02 ^a	0.86 ± 0.04 ^{ab}
RLW (g 100 g ⁻¹ BW)	1	3.89 ± 0.29 ^a	4.28 ± 0.02 ^c	4.61 ± 0.03 ^d	4.47 ± 0.03 ^d	4.21 ± 0.01 ^c	3.97 ± 0.04 ^a	3.94 ± 0.12 ^a
	7	3.93 ± 0.28 ^a	4.08 ± 0.05 ^{a*}	4.18 ± 0.02 ^{a*}	3.99 ± 0.01 ^{a*}	3.89 ± 0.01 ^{a*}	3.91 ± 0.03 ^a	3.87 ± 0.08 ^a
	14	3.92 ± 0.14 ^a	4.04 ± 0.03 ^{a*}	4.08 ± 0.06 ^{a*}	3.98 ± 0.01 ^{a*}	3.87 ± 0.02 ^{a*}	3.90 ± 0.13 ^a	3.86 ± 0.03 ^a
RKW (g 100 g ⁻¹ BW)	1	0.92 ± 0.02 ^a	1.01 ± 0.01 ^c	1.08 ± 0.001 ^d	1.00 ± 0.02 ^c	0.95 ± 0.003 ^b	0.95 ± 0.01 ^a	0.94 ± 0.01 ^a
	7	0.90 ± 0.02 ^a	0.91 ± 0.004 ^{a*}	0.97 ± 0.001 ^{b*}	0.97 ± 0.01 ^b	0.92 ± 0.01 ^{a*}	0.92 ± 0.01 ^a	0.92 ± 0.01 ^a
	14	0.91 ± 0.02 ^a	0.88 ± 0.004 ^{a*}	0.92 ± 0.001 ^{a*}	0.94 ± 0.01 ^{a*}	0.88 ± 0.01 ^{a*}	0.91 ± 0.03 ^a	0.92 ± 0.02 ^a
FC (g rat ⁻¹ day ⁻¹)	1	21.28 ± 0.37 ^c	17.66 ± 0.25 ^c	15.80 ± 0.20 ^a	16.57 ± 0.17 ^b	19.15 ± 0.10 ^d	18.83 ± 0.20 ^d	19.41 ± 0.18 ^d
	7	21.31 ± 0.29 ^c	18.79 ± 0.45 ^{b*}	17.89 ± 0.30 ^{ab*}	18.52 ± 0.19 ^{ab*}	20.96 ± 0.21 ^{c*}	20.52 ± 0.35 ^c	19.25 ± 0.45 ^c
	14	21.39 ± 0.39 ^c	21.33 ± 0.33 ^{c*#}	19.34 ± 0.19 ^{a*#}	19.94 ± 0.19 ^{ab*#}	21.78 ± 0.23 ^{c*#}	20.11 ± 0.07 ^c	21.94 ± 0.18 ^c
WC (mL rat ⁻¹ day ⁻¹)	1	22.67 ± 0.41 ^a	26.20 ± 0.11 ^b	27.65 ± 0.25 ^c	27.45 ± 0.30 ^c	25.00 ± 0.36 ^b	25.60 ± 0.32 ^b	25.44 ± 0.76 ^b
	7	23.00 ± 0.56 ^{ab}	24.29 ± 0.10 ^{b*}	26.90 ± 0.19 ^{c*}	26.23 ± 0.23 ^{c*}	23.31 ± 0.67 ^{ab*}	22.53 ± 0.73 ^{a*}	22.60 ± 0.81 ^{ab*}
	14	22.93 ± 0.83 ^a	22.67 ± 0.18 ^{a*#}	24.40 ± 0.32 ^{b*#}	25.15 ± 0.30 ^{b*#}	22.07 ± 0.24 ^{a*#}	22.67 ± 0.48 ^{a*}	22.64 ± 0.69 ^{a*}

Data is represented as mean ± standard error of mean

In the same row, the values marked with the same superscript letter are similar ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

“a”, “*”, “#” and “#” are significantly different (at $\alpha = 0.05$), as compared with the values at the zero, first, and seventh days, respectively

I: control rats; II: rats administered 0.5 g NiO-NPs; III: rats administered 1.0 g NiO-NPs; IV: rats administered 0.5 g Co₃O₄-NPs; V: rats administered 1.0 g Co₃O₄-NPs; VI: rats administered 0.25 g NiO-NPs + 0.25 g Co₃O₄-NPs; and VII: rats administered 0.5 g NiO-NPs + 0.5 g Co₃O₄-NPs

or/and Co₃O₄-NPs showed marked reductions in the FC accompanied by remarkable elevations in the WC, as compared with the controls. At all experimental times, by increasing the administered dose of NiO-NPs in rats administered NiO-NPs alone, FC was significantly decreased, whereas WC was markedly increased. On the contrary, in rats administered Co₃O₄-NPs alone, by increasing the dose of Co₃O₄-NPs, FC was remarkably elevated, but WC was significantly reduced, at all intervals. In rats administered NiO-NPs or Co₃O₄-NPs, fluctuations in FC and WC were time-dependent. By the seventh and fourteenth days, FC and WC of rats of groups VI and VII were similar to the controls.

Effect of nanoparticles on the hematological parameters

In Table 2, the hematological parameters including WBC and RBC counts, Hb content, and PCV of all the experimental rats were recorded. In rats administered with NiO-NPs or Co₃O₄-NPs, WBC count was remarkably lower than it in group I, at all the experimental intervals, except at the fourteenth day in groups II and V. By the fourteenth day, WBC count of rats administered NiO-NPs or Co₃O₄-NPs became markedly higher than at the first day. The WBC count of the rats administered with NiO-NPs in combination with Co₃O₄-NPs showed similarity to the controls, at most experimental durations. At the

first day, all the experimental groups showed significant elevations in RBC count, except in groups VI and VII. By the seventh and fourteenth days, RBC count of all groups was similar to that in group I, except for marked elevations in group III (at the seventh and fourteenth days) and group IV (at the seventh day). At most time intervals, RBC count of rats administered NiO-NPs or Co₃O₄-NPs was significantly reduced, as compared with the first day. The RBC count of rats administered NiO-NPs or Co₃O₄-NPs was markedly increased with increasing NiO-NPs dose (at the first and seventh days), while it decreased with increasing Co₃O₄-NPs dose (at the first day). The Hb contents of all groups were insignificantly differed among all the experimental groups, except for significant elevations at the first day in groups III and IV. At all intervals, rats administered with NiO-NPs or Co₃O₄-NPs separately showed remarkable elevations in PCV, as compared with the group I. By the fourteenth day, PCVs of rats administered NiO-NPs or Co₃O₄-NPs were markedly lower than at the first day. In comparison with group I, rats administered NiO-NPs and Co₃O₄-NPs together did not exhibit any significant changes in PCV, throughout the experiments, except for marked elevations in group VI (at the first and seventh days) and in group VII (at the first day). In rats administered NiO-NPs or Co₃O₄-NPs, PCVs were elevated with increasing NiO-NPs dose, whereas these reduced with increasing Co₃O₄-NPs levels, on the first and seventh days.

Table 2 The white and red blood cells (WBC and RBC) counts, hemoglobin (Hb) content, and packed cell volume (PCV) of all the experimental groups, throughout the experiments

Parameter	Time (day)	Experimental group						
		I	II	III	IV	V	VI	VII
WBCs ($\times 10^9 L^{-1}$)	1	11.42 \pm 0.17 ^c	9.90 \pm 0.61 ^{ab}	9.26 \pm 0.24 ^a	9.39 \pm 0.44 ^{ab}	9.92 \pm 0.37 ^{ab}	9.97 \pm 0.33 ^{ab}	10.47 \pm 0.25 ^{bc}
	7	11.52 \pm 0.16 ^d	10.58 \pm 0.44 ^c	9.33 \pm 0.36 ^a	9.46 \pm 0.13 ^{ab}	10.24 \pm 0.10 ^{bc}	10.63 \pm 0.20 ^c	11.44 \pm 0.32 ^d
	14	11.55 \pm 0.20 ^b	10.95 \pm 0.20 ^{ab*}	10.01 \pm 0.20 ^{a*}	10.18 \pm 0.18 ^{a*}	10.76 \pm 0.61 ^{ab*}	10.95 \pm 0.49 ^{ab}	11.55 \pm 0.35 ^b
RBCs ($\times 10^{12} L^{-1}$)	1	6.82 \pm 0.18 ^a	8.32 \pm 0.12 ^b	8.89 \pm 0.12 ^c	8.99 \pm 0.31 ^c	8.26 \pm 0.07 ^b	7.81 \pm 0.13 ^a	7.20 \pm 0.33 ^a
	7	6.87 \pm 0.10 ^a	7.37 \pm 0.06 ^{ab*}	8.38 \pm 0.08 ^c	7.75 \pm 0.36 ^{bc}	7.12 \pm 0.27 ^{ab*}	7.44 \pm 0.42 ^{ab}	6.97 \pm 0.27 ^{ab}
	14	6.81 \pm 0.28 ^a	7.22 \pm 0.27 ^{ab*}	7.68 \pm 0.13 ^{b*}	7.47 \pm 0.21 ^{ab*}	6.88 \pm 0.28 ^{ab*}	6.89 \pm 0.30 ^{ab}	6.81 \pm 0.29 ^a
Hb (g dL ⁻¹)	1	13.51 \pm 0.38 ^a	14.03 \pm 0.26 ^a	15.27 \pm 0.21 ^b	15.12 \pm 0.26 ^b	14.03 \pm 0.14 ^a	14.07 \pm 0.26 ^a	13.89 \pm 0.27 ^a
	7	13.42 \pm 0.57 ^a	13.83 \pm 0.45 ^a	14.42 \pm 0.29 ^{a*}	14.23 \pm 0.16 ^{a*}	13.86 \pm 0.14 ^a	13.95 \pm 0.53 ^a	13.84 \pm 0.29 ^a
	14	13.66 \pm 0.21 ^a	13.57 \pm 0.39 ^a	13.64 \pm 0.23 ^{a*}	13.72 \pm 0.31 ^{a*}	13.57 \pm 0.13 ^a	13.79 \pm 0.29 ^a	13.71 \pm 0.27 ^a
PCV (%)	1	43.19 \pm 0.29 ^a	50.68 \pm 0.38 ^{de}	53.85 \pm 0.31 ^f	51.77 \pm 1.90 ^{ef}	49.19 \pm 0.62 ^{cd}	48.12 \pm 0.56 ^{bc}	46.29 \pm 0.31 ^b
	7	43.27 \pm 0.34 ^a	48.53 \pm 0.78 ^{cd}	51.31 \pm 0.51 ^e	50.59 \pm 0.12 ^{de}	46.41 \pm 0.31 ^{bc*}	47.36 \pm 1.80 ^{bc}	45.28 \pm 0.99 ^{ab}
	14	43.09 \pm 0.58 ^a	46.31 \pm 0.32 ^{b*}	46.87 \pm 0.55 ^{b*#}	47.01 \pm 1.17 ^{b*#}	46.38 \pm 0.40 ^{b*}	43.83 \pm 0.40 ^a	43.64 \pm 0.24 ^a

In the same raw, the values marked with the same superscript letter are similar ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

“*” and “#” are significantly different (at $\alpha = 0.05$), as compared with the values at the first and seventh days, respectively

Data is represented as mean \pm standard error of mean

I: Control rats; II: rats administered 0.5 g NiO-NPs; III: rats administered 1.0 g NiO-NPs; IV: rats administered 0.5 g Co₃O₄-NPs; V: rats administered 1.0 g Co₃O₄-NPs; VI: rats administered 0.25 g NiO-NPs + 0.25 g Co₃O₄-NPs; and VII: rats administered 0.5 g NiO-NPs + 0.5 g Co₃O₄-NPs

Table 3 The levels of total proteins (TP), albumin, globulin, and albumin/globulin ratio of all the experimental groups, throughout the experiments

Parameter	Time (day)	Experimental groups						
		I	II	III	IV	V	VI	VII
TP (g dL ⁻¹)	1	6.74 \pm 0.06 ^f	5.95 \pm 0.04 ^{cd}	5.04 \pm 0.01 ^a	5.64 \pm 0.02 ^b	6.17 \pm 0.03 ^{de}	5.84 \pm 0.17 ^{bc}	6.37 \pm 0.13 ^e
	7	6.68 \pm 0.07 ^d	6.17 \pm 0.09 ^{bc}	5.92 \pm 0.11 ^{a*}	6.05 \pm 0.10 ^{ab*}	6.46 \pm 0.07 ^{cd*}	6.37 \pm 0.17 ^{cd*}	6.43 \pm 0.13 ^{cd}
	14	6.65 \pm 0.05 ^a	6.64 \pm 0.25 ^{a*}	6.47 \pm 0.02 ^{a*}	6.56 \pm 0.21 ^{a*}	6.57 \pm 0.05 ^{a*}	6.56 \pm 0.21 ^{a*}	6.69 \pm 0.16 ^a
Albumin (g dL ⁻¹)	1	4.06 \pm 0.02 ^f	3.33 \pm 0.01 ^c	2.47 \pm 0.04 ^a	3.09 \pm 0.02 ^b	3.52 \pm 0.05 ^d	3.31 \pm 0.13 ^c	3.83 \pm 0.07 ^e
	7	4.04 \pm 0.01 ^d	3.61 \pm 0.02 ^{bc}	3.33 \pm 0.03 ^{a*}	3.43 \pm 0.02 ^{ab*}	3.82 \pm 0.05 ^{d*}	3.77 \pm 0.12 ^{cd*}	3.87 \pm 0.08 ^d
	14	4.02 \pm 0.01 ^a	3.99 \pm 0.02 ^{a*}	3.87 \pm 0.05 ^{a*}	3.94 \pm 0.04 ^{a*}	3.99 \pm 0.03 ^{a*}	3.92 \pm 0.01 ^{a*}	4.02 \pm 0.02 ^a
Globulin (g dL ⁻¹)	1	2.67 \pm 0.05 ^a	2.63 \pm 0.03 ^a	2.57 \pm 0.04 ^a	2.54 \pm 0.01 ^a	2.65 \pm 0.02 ^a	2.52 \pm 0.10 ^a	2.55 \pm 0.06 ^a
	7	2.64 \pm 0.06 ^a	2.57 \pm 0.08 ^a	2.59 \pm 0.09 ^a	2.62 \pm 0.08 ^a	2.65 \pm 0.03 ^a	2.60 \pm 0.04 ^a	2.57 \pm 0.09 ^a
	14	2.62 \pm 0.05 ^a	2.64 \pm 0.24 ^a	2.60 \pm 0.04 ^a	2.62 \pm 0.17 ^a	2.59 \pm 0.02 ^a	2.64 \pm 0.21 ^a	2.67 \pm 0.14 ^a
Albumin/globulin ratio	1	1.52 \pm 0.02 ^d	1.27 \pm 0.01 ^{bc}	0.96 \pm 0.03 ^a	1.22 \pm 0.01 ^b	1.33 \pm 0.03 ^c	1.32 \pm 0.08 ^c	1.40 \pm 0.02 ^c
	7	1.53 \pm 0.03 ^d	1.41 \pm 0.04 ^{bc}	1.29 \pm 0.04 ^{a*}	1.31 \pm 0.04 ^{ab*}	1.44 \pm 0.02 ^{cd*}	1.45 \pm 0.03 ^{cd*}	1.51 \pm 0.05 ^{cd}
	14	1.54 \pm 0.03 ^a	1.55 \pm 0.12 ^{a*}	1.49 \pm 0.04 ^{a*}	1.53 \pm 0.09 ^{a*}	1.54 \pm 0.01 ^{a*}	1.53 \pm 0.13 ^{a*}	1.52 \pm 0.08 ^a

Data is represented as mean \pm standard error of mean

In the same raw, the values marked with the same superscript letter are similar ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

“*” and “#” are significantly different (at $\alpha = 0.05$), as compared with the values at the first and seventh days, respectively

I: Control rats; II: rats administered 0.5 g NiO-NPs; III: rats administered 1.0 g NiO-NPs; IV: rats administered 0.5 g Co₃O₄-NPs; V: rats administered 1.0 g Co₃O₄-NPs; VI: rats administered 0.25 g NiO-NPs + 0.25 g Co₃O₄-NPs; and VII: rats administered 0.5 g NiO-NPs + 0.5 g Co₃O₄-NPs

Effect of nanoparticles on the serum biochemical parameters

Effect on serum protein profile

The serum protein profile of all the experimental rats was shown in Table 3. At the first day, serum levels of TP, albumin, and albumin/globulin ratio of all groups were significantly declined, as compared with group I. By the seventh day, groups II, III, and IV exhibited marked depletions in all protein variables, except globulin levels, as compared with the controls. On the fourteenth day, all protein parameters of rats administered NiO-NPs or/and Co₃O₄-NPs were similar to group I. The levels of TP, albumin, and albumin/globulin ratio of rats administered NiO-NPs or Co₃O₄-NPs were remarkably decreased by increasing NiO-NPs dose, whereas these were significantly increased with elevating Co₃O₄-NPs dose, on the first and seventh days.

Effect on serum lipid profile

In Table 4, the serum levels of TC, TG, HDL-C, and LDL-C of control rats and those administered with NiO-NPs or/and Co₃O₄-NPs were displayed. At all experimental intervals, rats administered with NiO-NPs or Co₃O₄-NPs as well as rats of group VI showed remarkable declines in serum levels of TC,

TG, and HDL-C, whereas marked elevations in LDL-C levels, in comparison with controls, were observed. In group VII, significant reductions in TC, TG, and HDL-C as well as marked elevation in LDL-C were recorded at the first and seventh days only. At most experimental periods, in rats administered NiO-NPs or Co₃O₄-NPs, TC, TG, and HDL-C were markedly increased with increasing Co₃O₄-NPs doses, while they were decreased with increasing NiO-NPs dose. On the other hand, LDL-C levels of rats administered NiO-NPs or Co₃O₄-NPs were increased by increasing the administered dose of NiO-NPs, but these were markedly depleted with increasing dose of Co₃O₄-NPs, at most experimental durations.

Effect on liver and renal functions as well as glucose levels in serum

The activities of ASAT and ALAT as well as the levels of glucose, creatinine, urea, and uric acid in serum of all the experimental rats were demonstrated in Table 5. In the rats administered with NiO-NPs or Co₃O₄-NPs, there were marked elevations in the activities of ASAT and ALAT, as compared with the group I, at most time intervals. The activities of ASAT and ALAT of groups II, III, IV, and V were markedly declined by increasing time. On the first day, the activities of ASAT and ALAT of groups II were significantly lower than in group III. On the other hand, in group IV, the

Table 4 The levels of total cholesterol (TC), triglycerides (TG), low- and high-density lipoprotein cholesterol (LDL-C & HDL-C) of all the experimental groups, throughout the experiments

Parameter	Time (day)	Experimental group						
		I	II	III	IV	V	VI	VII
TC (mg dL ⁻¹)	1	118.09 ± 1.26 ^d	94.42 ± 2.06 ^c	69.44 ± 2.12 ^a	64.98 ± 2.62 ^a	87.82 ± 2.58 ^b	94.91 ± 0.47 ^c	99.50 ± 0.44 ^c
	7	118.25 ± 0.63 ^c	103.76 ± 1.31 ^{c*}	98.91 ± 1.21 ^{b*}	92.86 ± 1.10 ^{a*}	102.20 ± 1.15 ^{c*}	101.66 ± 0.56 ^{b*}	111.38 ± 0.73 ^{d*}
	14	118.14 ± 0.38 ^d	113.27 ± 0.52 ^{c*}	102.41 ± 0.69 ^{a*}	102.76 ± 0.64 ^{a*}	112.02 ± 1.97 ^{c*}	109.40 ± 0.45 ^{b*}	117.22 ± 0.58 ^{d*}
TG (mg dL ⁻¹)	1	89.01 ± 3.57 ^c	45.56 ± 0.41 ^b	38.86 ± 0.21 ^a	35.28 ± 0.53 ^a	49.63 ± 0.56 ^b	63.72 ± 3.20 ^c	73.71 ± 3.45 ^d
	7	88.88 ± 2.40 ^c	59.02 ± 0.33 ^{b*}	50.64 ± 0.24 ^{a*}	58.99 ± 0.27 ^{b*}	64.13 ± 0.35 ^{c*}	64.84 ± 1.56 ^c	81.96 ± 1.33 ^d
	14	88.96 ± 3.72 ^d	77.97 ± 0.71 ^{bc*#}	64.25 ± 0.43 ^{a*#}	66.14 ± 1.19 ^{a*#}	74.22 ± 1.03 ^{b*#}	77.65 ± 2.62 ^{b*}	85.23 ± 1.83 ^d
LDL-C (mg dL ⁻¹)	1	62.33 ± 1.17 ^a	66.96 ± 1.39 ^b	73.70 ± 0.83 ^c	74.38 ± 0.65 ^c	67.35 ± 1.63 ^b	68.48 ± 0.28 ^b	65.22 ± 0.29 ^b
	7	61.33 ± 1.07 ^a	65.57 ± 0.56 ^b	70.23 ± 0.64 ^c	70.22 ± 1.21 ^c	64.52 ± 0.84 ^b	66.95 ± 0.58 ^b	64.47 ± 0.67 ^b
	14	62.01 ± 0.71 ^a	64.02 ± 0.72 ^{ab}	68.54 ± 0.56 ^c	66.54 ± 0.39 ^{bc}	64.90 ± 2.88 ^{bc}	65.06 ± 0.35 ^{bc}	62.14 ± 0.66 ^a
HDL-C (mg dL ⁻¹)	1	31.10 ± 1.42 ^c	19.39 ± 1.30 ^b	15.23 ± 0.77 ^a	18.34 ± 0.71 ^b	22.70 ± 0.63 ^c	24.24 ± 0.20 ^c	27.55 ± 0.83 ^d
	7	30.56 ± 0.85 ^c	20.58 ± 1.07 ^b	17.38 ± 0.78 ^{a*}	20.37 ± 0.11 ^{b*}	22.66 ± 0.42 ^c	28.16 ± 0.28 ^{d*}	29.48 ± 0.35 ^d
	14	30.91 ± 1.23 ^b	22.97 ± 0.61 ^a	22.54 ± 0.63 ^{a*#}	21.69 ± 0.61 ^{a*}	23.80 ± 0.48 ^a	28.60 ± 0.52 ^{a*}	31.97 ± 0.61 ^{b*}

Data is represented as mean ± standard error of mean

In the same raw, the values marked with the same superscript letter are similar ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

“*” and “#” are significantly different (at $\alpha = 0.05$), as compared with the values at the first and seventh days, respectively

I: Control rats; II: rats administered 0.5 g NiO-NPs; III: rats administered 1.0 g NiO-NPs; IV: rats administered 0.5 g Co₃O₄-NPs; V: rats administered 1.0 g Co₃O₄-NPs; VI: rats administered 0.25 g NiO-NPs + 0.25 g Co₃O₄-NPs; and VII: rats administered 0.5 g NiO-NPs + 0.5 g Co₃O₄-NPs.

Table 5 The activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) as well as levels of glucose, creatinine, urea, and uric acid of all the experimental groups, throughout the experiments

Parameter	Time (day)	Experimental group						
		I	II	III	IV	V	VI	VII
ASAT (U L ⁻¹)	1	35.47 ± 0.90 ^a	51.56 ± 0.53 ^d	58.82 ± 0.77 ^c	59.09 ± 0.81 ^c	48.49 ± 0.82 ^c	41.07 ± 0.74 ^b	40.48 ± 0.24 ^b
	7	35.24 ± 0.96 ^a	42.87 ± 0.71 ^{d*}	47.04 ± 0.49 ^{bc*}	45.24 ± 0.27 ^{c*}	41.63 ± 0.16 ^{c*}	40.28 ± 0.32 ^b	39.05 ± 0.28 ^a
	14	35.11 ± 0.87 ^a	39.48 ± 0.56 ^{b*#}	42.91 ± 0.27 ^{c*#}	41.51 ± 0.48 ^{c*#}	38.06 ± 0.35 ^{b*#}	35.34 ± 0.26 ^{a*#}	34.92 ± 0.58 ^{a*#}
ALAT (U L ⁻¹)	1	24.03 ± 0.48 ^a	27.02 ± 0.38 ^b	31.18 ± 0.56 ^d	29.00 ± 0.37 ^c	25.52 ± 0.53 ^b	25.89 ± 0.08 ^b	25.73 ± 1.01 ^b
	7	23.25 ± 0.34 ^a	25.55 ± 0.49 ^c	27.25 ± 0.49 ^d	25.80 ± 0.27 ^{c*}	24.03 ± 0.26 ^b	25.35 ± 0.68 ^c	25.10 ± 0.31 ^{bc}
	14	23.32 ± 0.33 ^a	25.34 ± 0.43 ^b	26.37 ± 0.29 ^{c*}	25.12 ± 0.25 ^{b*}	23.87 ± 0.16 ^{a*}	23.50 ± 0.32 ^{a*}	23.44 ± 0.44 ^{a*}
Glucose (mg dL ⁻¹)	1	75.41 ± 4.82 ^a	98.08 ± 0.91 ^{bc}	124.69 ± 1.31 ^d	105.07 ± 1.53 ^c	95.44 ± 0.46 ^b	94.53 ± 3.80 ^b	94.28 ± 2.53 ^b
	7	79.43 ± 2.53 ^a	83.84 ± 1.21 ^{b*}	97.60 ± 0.98 ^{d*}	88.56 ± 0.70 ^{c*}	82.63 ± 0.46 ^{ab*}	82.41 ± 0.12 ^{ab*}	81.37 ± 1.49 ^{ab*}
	14	77.40 ± 4.37 ^a	77.51 ± 0.76 ^{a*#}	88.86 ± 1.67 ^{b*#}	85.42 ± 0.97 ^{b*}	76.52 ± 0.72 ^{a*}	79.67 ± 2.04 ^{a*}	78.13 ± 2.10 ^{a*}
Creatinine (mg dL ⁻¹)	1	1.46 ± 0.01 ^a	1.53 ± 0.01 ^b	1.63 ± 0.02 ^c	1.53 ± 0.01 ^b	1.47 ± 0.01 ^a	1.48 ± 0.003 ^a	1.48 ± 0.01 ^a
	7	1.45 ± 0.01 ^a	1.48 ± 0.01 ^{bc}	1.54 ± 0.01 ^{d*}	1.51 ± 0.01 ^c	1.45 ± 0.01 ^{ab}	1.46 ± 0.01 ^{ab*}	1.44 ± 0.01 ^a
	14	1.45 ± 0.02 ^a	1.43 ± 0.01 ^{a*}	1.45 ± 0.01 ^{a*#}	1.46 ± 0.01 ^{a*}	1.43 ± 0.01 ^a	1.45 ± 0.01 ^{a*}	1.44 ± 0.01 ^a
Urea (mg dL ⁻¹)	1	35.85 ± 0.46 ^a	55.93 ± 1.23 ^c	70.83 ± 1.42 ^c	70.10 ± 0.67 ^c	59.17 ± 0.65 ^d	53.66 ± 0.92 ^c	47.85 ± 0.73 ^b
	7	35.69 ± 0.78 ^a	51.98 ± 1.65 ^c	55.76 ± 0.19 ^{d*}	56.80 ± 0.57 ^{d*}	47.22 ± 0.69 ^{b*}	50.82 ± 0.50 ^c	46.81 ± 0.41 ^b
	14	35.97 ± 0.46 ^a	43.42 ± 0.95 ^c	53.90 ± 0.85 ^{d*}	42.99 ± 0.46 ^{c*#}	37.56 ± 0.65 ^{a*#}	36.26 ± 0.72 ^{a*#}	36.84 ± 0.54 ^{a*#}
Uric acid (mg dL ⁻¹)	1	1.44 ± 0.01 ^a	2.55 ± 0.05 ^c	3.23 ± 0.08 ^c	2.81 ± 0.07 ^d	2.73 ± 0.11 ^{cd}	2.28 ± 0.02 ^b	2.10 ± 0.03 ^b
	7	1.45 ± 0.02 ^a	2.39 ± 0.06 ^d	2.42 ± 0.03 ^{d*}	2.44 ± 0.08 ^{d*}	2.12 ± 0.06 ^{c*}	2.11 ± 0.04 ^{c*}	1.94 ± 0.02 ^{b*}
	14	1.46 ± 0.03 ^a	1.88 ± 0.03 ^{b*#}	2.12 ± 0.03 ^{c*#}	2.20 ± 0.18 ^{c*#}	1.64 ± 0.01 ^{a*#}	1.49 ± 0.01 ^{a*#}	1.44 ± 0.01 ^{a*#}

Data is represented as mean ± standard error of mean

In the same raw, the values marked with the same superscript letter are similar ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

“*” and “#” are significantly different (at $\alpha = 0.05$), as compared with the values at the first and seventh days, respectively

I: Control rats; II: rats administered 0.5 g NiO-NPs; III: rats administered 1.0 g NiO-NPs; IV: rats administered 0.5 g Co₃O₄-NPs; V: rats administered 1.0 g Co₃O₄-NPs; VI: rats administered 0.25 g NiO-NPs + 0.25 g Co₃O₄-NPs; and VII: rats administered 0.5 g NiO-NPs + 0.5 g Co₃O₄-NPs

activities of serum transaminases were remarkably higher than in group V. In rats administered NiO-NPs in association with Co₃O₄-NPs, at the first and seventh days, the transaminases activities were significantly higher than in group I, except for insignificant change in ASAT activity of group VII. By the fourteenth day, ASAT and ALAT activities of groups VI and VII were returned to the control values.

At most intervals, glucose levels of rats administered with NiO-NPs or Co₃O₄-NPs solely were significantly increased, as compared with group I. The creatinine levels of all groups did not exhibit any marked differences with those of group I except in groups II, III, and IV at the first and seventh days. Serum levels of urea and uric acid of rats of groups II, III, IV, and V were significantly higher than in group I, at most experimental periods. Rats treated individually with either NiO-NPs or Co₃O₄-NPs showed significant elevations in glucose, creatinine, urea, and uric acid with increasing the administered dose of NiO-NPs as well as with decreasing the dose of Co₃O₄-NPs. Glucose and creatinine levels of groups VI and VII were similar to the controls at all times, except for marked elevations in glucose levels at the first day in both groups. Rats

of groups VI and VII showed significant increase in urea and uric acid levels at the first and seventh days, as compared with the controls.

Discussion

The present study is a continuation and follow-up of two recent researches about the combined actions of NiO-NPs and Co₃O₄-NPs in rats. The first study was designed to characterize NiO-NPs and Co₃O₄-NPs, estimate their median lethal doses, and follow up the accumulation patterns and toxicokinetics of their metal ions in tissues of rats (Ali 2018). In the second study, genotoxicity and oxidative damage induced by acute exposure to NiO-NPs and/or Co₃O₄-NPs were studied (Ali and Mohamed 2019). The objective of the current work was to evaluate the changes in biological, hematological, and biochemical parameters of rats administered NiO-NPs or/and Co₃O₄-NPs.

Herein, TBW of rats administered NiO-NPs or Co₃O₄-NPs was remarkably reduced at the first day, as compared with the

zero day. The recorded reduction of TBW can be linked to the reduced FC rates. Similarly, Kong et al. (2014) reported a marked reduction in the TBW of male rats after an exposure to Ni-NPs. The present data revealed significant elevations in RBW, RKW, and RLW of rats administered NiO-NPs or Co₃O₄-NPs, indicating the incidence of toxicity (Kim et al. 2007 and Wang et al. 2016). This can be ensured by the reported elevations in serum activities of aminotransferases as well as levels of creatinine, urea, and uric acid. It is noteworthy that the relative organ weights are favored than the absolute organ weights, because the former parameters account for the differences in the TBW (Mathuramon et al. 2009). Accordingly, a significant increment in RBW, RKW, and RLW can be ascribed to the reported decline of TBW. Moreover, the elevated relative organ weights may be due to the increased amount of water inside these tissues, as a result of the increased WC, to reduce the concentration of the toxic NPs. Magaye et al. (2014) also recorded a significant elevation in the RLW of rats following an intravenous injection with NiO-NPs. Chattopadhyay et al. (2015) found that the rats injected subcutaneously with 0.5 and 1.0 mg of CoO-NPs/kg BW showed marked elevations in the weights of the spleen and liver of mice accompanied by marked reduction in the TBW, as compared with the controls. The increased amount of WC can lead to faster excretion of the toxic NPs via the urine after passing through the fine glomerular pores (Dumala et al. 2018).

The obtained results showed a remarkable reduction in WBC count accompanied with marked elevations in RBC count, Hb content, and PCV of rats administered NiO-NPs or Co₃O₄-NPs. These findings can be considered as a mechanism to overcome the hypoxia induced by NP intoxication (Morsy et al. 2016). Hence, hypoxia activates erythropoietin secretion from renal tissue causing enhanced erythropoiesis (Magaye et al. 2014). The increased PCV indicates an elevated blood viscosity. High PCV can be generally linked to the abnormal increment of RBC production (Shah 2006). Similarly, Magaye et al. (2014) reported significant elevations in the number of erythrocytes as well as Hb levels in rats after intravenous injection with 20 mg of Ni-NPs/kg BW. On the other hand, the reported depletion in WBC count can be ascribed to the ability of NiO-NPs and Co₃O₄-NPs to cause cellular damage via excessive generation of reactive oxygen species (ROS) (Ali and Mohamed 2019). Moreover, the reduced WBC count may be linked to the deficiency in the proteins and lipids required for their synthesis (Morsy et al. 2016). This can be confirmed by the reduced TP, albumin, TC, TG, and HDL-C.

The acute administration of NiO-NPs or Co₃O₄-NPs caused remarkable reductions in serum levels of TP and albumin, whereas insignificant change in globulin level was observed. Thus, the albumin/globulin ratio was markedly reduced. These findings may be attributed to an excessive utilization of albumin as an antioxidant against the deleterious

effects of the oxidative stress induced by the chemicals (Tolia et al. 2013). Amirtharaj et al. (2008) documented the ability of the albumin to capture the Ni and Co ions. Furthermore, the reported hypoalbuminemia may be due to an impaired protein synthesis as a result of NP-induced hepatocellular damage. This was ensured by the increased activities of the ASAT and ALAT. Moreover, the reduced levels of TP and albumin may be attributed to the ability of NiO-NPs and Co₃O₄-NPs to induce significant damages on gene level, by the interaction of the ROS with DNA structures (Ali and Mohamed 2019), leading to suppression in the protein biosynthesis (Morsy et al. 2016). Moreover, NPs may induce direct degradations to the plasma proteins via activation of endopeptidases (Morsy et al. 2016). In addition, the reported reductions in protein levels can be attributed to the leakage of albumin and amino acids from the renal tubules as a result of kidney dysfunction (Saad et al. 2016). This was confirmed by significant elevations in the levels of creatinine, urea, and uric acid.

In the present data, rats administered NiO-NPs or Co₃O₄-NPs showed significant reductions in serum levels of the TC, TG, and HDL-C, whereas remarkable increase in LDL-C level. The reduced levels of the TG can be related to the immense utilization of them as energy supply for the cells to withstand toxicity. The LDL-C is responsible for the transport of the cholesterol to the blood, whereas the HDL-C moves it back to the liver (Abdel-Ghaffar et al. 2018). Accordingly, an increase in the TC level was expected to occur. However, a momentous decline was recorded in the TC levels which were recorded after the treatment with any of the NiO-NPs and Co₃O₄-NPs. These findings can be attributed to the ability of the cobalt and nickel oxide NPs to induce lipid peroxidation as a result of ROS overproduction (Ali and Mohamed 2019). Furthermore, the NPs can induce an oversecretion of catecholamine leading to an excessive oxidation of the lipids for energy production (Morsy et al. 2016). The increased excretion of the lipids via the kidney can be another cause of the reduced levels of TC and TG, as a result of impaired renal functions.

Glucose is considered the prime source of energy for cellular activities under stress. The increased levels of glucose may be attributed to the enhanced breakdown of glycogen stores in the tissues (Belanger et al. 2011) as well as due to the formation of glucose by gluconeogenesis (Shaikh and Desai 2016). It is well known that the activation of the insulin receptors involves the dephosphorylation of their serine-threonine residues to allow glucose uptake by cells (Lukic et al. 2014). However, NPs can induce phosphorylation of serine-threonine residues via ROS that activates serine-threonine kinases, resulting in an insulin resistance and subsequent glucose accumulation in the blood (Hu et al. 2015). Similarly, Shaikh and Desai (2016) observed a significant increase in blood glucose levels of mice after an oral intake of cobalt oxide NPs. In the same line, Hu et al. (2015) reported a

significant elevation in plasma levels of glucose after treatment of mice with titanium oxide NPs.

The present results revealed remarkable elevations in the ASAT and ALAT activities, after the exposure to NiO-NPs or Co₃O₄-NPs. These findings can be linked to the ability of the liver to accumulate the NPs (Ali 2018), resulting in an altered permeability of the cellular membranes (Yang et al. 2008). In the same line, Dumala et al. (2017) reported remarkable elevations in the activities of ASAT and ALAT, in female Wister albino rats after an acute oral exposure to 500 mg of NiO-NPs/kg BW. They attributed it to the ability of NPs to induce necrosis in the liver cells due to the accumulation of these NPs in the liver.

In the present results, significant elevations in the concentrations of the creatinine, urea, and uric acid in the rats exposed to NiO-NPs or Co₃O₄-NPs can be attributed to the ability of the renal tissue to accumulate a significant amount of xenobiotic for further excretion (Ibraheem et al. 2016). This was ensured by Ali (2018) who reported significant increase in the levels of accumulated Ni and Co in renal tissue of rats administered NiO-NPs or Co₃O₄-NPs. In the muscle, the catabolism of creatine phosphate that acts as energy reservoir results in creatinine (Morsy et al. 2016). The elevated serum levels of creatinine can also indicate an impaired glomerular filtration rate due to the expansion of the vascular glomeruli under metal toxicity (Abdel Aziz and Zabut 2011). The increased urea levels reflect an increased breakdown of protein as confirmed by the reduced TP content. Moreover, the Dumala et al. (2018) suggested that the accumulation of NiO-NPs can disturb the cellular structure of the renal tissue causing the damage of their membranes. Accordingly, this can impede the filtration of the uric acid, urea, and creatinine leading to their buildup in the blood.

The administration of the NiO-NPs and Co₃O₄-NPs separately caused remarkable disturbances in nearly all the studied variables. On the other hand, the combined administration of NiO-NPs with Co₃O₄-NPs caused less significant defects in all the studied parameters. These findings were in accordance with the accumulation patterns of NiO-NPs and Co₃O₄-NPs in the liver and kidney of rats (Ali 2018). Likewise, Ibraheem et al. (2016) found that the combined administration of cadmium and aluminum to the mice had less destructive impact on the liver and kidney than their individual effects. Several mechanisms can explain these findings based on the alternations of their absorption, distribution, accumulation, and elimination. The liberated metal ions after the ionization of NPs can be responsible for their induced toxicity (Ates et al. 2016). Thus, it could be assumed that the NiO-NPs and Co₃O₄-NPs markedly reduced the ionization rates of each other (Ali 2018). Accordingly, the amount of ions that reach the blood will be declined. In the blood, the Ni and Co share the same carrier which is the albumin (Amirtharaj et al. 2008). Thus, the two ions may compete with each other on the binding sites of

the transporters. Once reached to the target organs, the repulsion between the two cations may impede their uptake by the cells. Therefore, the accumulation of these metal ions can be reduced. Similarly, He et al. (2015) reported that after 14 days of exposing *Enchytraeus crypticus* to a mixture of NiCl₂ and CoCl₂, the Ni was remarkably reduced in the presence of Co. The authors linked that to the similarity between the two divalent cations in their physicochemical properties leading to an antagonistic interaction on their transporting sites. Finally, the two ions can accelerate the excretion of each other (Ali 2018). This can be induced via increment of the glomerular filtration rates as ensured by the increased levels of serum creatinine, in the current work.

Conclusion

The present work revealed that the combined acute oral administration of the NiO-NPs and Co₃O₄-NPs to the male albino rats led to less remarkable disturbances in all the studied biological, hematological, and biochemical variables, as compared with their individual effects. Accordingly, it is recommended to use mixtures of NiO-NPs and Co₃O₄-NPs in their widely distributed applications, rather than their individual NPs.

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Compliance with ethical standards

Ethical approval The protocol of the present study was approved, at November 2017, by Cairo University—Institutional Animal Care and Use Committee (CU-IACUC), Giza, Egypt. The approval number was CU/I/F/97/17. All the rats were handled according to the international guidelines of the laboratory animal care and use.

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