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The influence of five metallic nanoparticles on the expression of major drug-metabolizing enzyme genes with correlation of inflammation in mouse livers

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ABSTRACT

Metallic nanoparticles (NPs) are widely used in medical preparations. The present study aims to find out the influence of widely used five metallic NPs on the expression of major hepatic drug-metabolizing enzyme (DME) genes. Six groups of BALB/C mice, 7 mice each, were exposed to: Gold NPs, silver NPs, copper oxide NPs, silicon dioxide NPs and zinc oxide NPs, for 21 days. Liver biopsies from all mice were subjected to mouse *cyp3a11*, *cyp2c29*, *ugt2b1* and *interleukin-6 (il6)* gene expression quantification using real-time polymerase chain reaction, in addition to inflammatory cell infiltration examination. All tested NPs caused a sharp and significant (ANOVA, p value < 0.05) downregulation in the expression of DME genes, with the highest influence was observed in mice exposed to copper oxide NPs. Additionally, all NPs induced hepatic inflammation and upregulated the expression of *il6* gene, which were inversely correlated with the expression of DMEs. It is concluded that all tested NPs downregulated the expression of DME genes, with the highest influence exhibited by copper oxide NPs, in correlation with inflammation and *il6* gene induction in the liver. Further studies are needed to find out the effect of anti-inflammatory compounds against the alterations induced by metallic NPs exposure on hepatic DMEs.

1. Introduction

Nanoparticles (NPs) are widely used in the industrial and pharmaceutical preparations (Sheth et al., 2012). Nanoparticles are generally classified into metallic and non-metallic with metallic ones can be pure or in the form of metallic oxide (Jeevanandam et al., 2018). Of the widely used metallic NPs are gold (Au) NPs, silver (Ag) NPs, copper oxide (CuO) NPs, zinc oxide (ZnO) NPs and silicon dioxide (SiO_2) NPs (Baptista et al., 2018).

Gold NPs are currently used in drug delivery, cancer therapy and *in vivo* imaging for biomedical applications (Peng et al., 2009). In addition, Au NPs hold a promise for many health disorders especially autoimmune diseases with Au NPs biosensor being used to detect lung cancer (Peng et al., 2009). On the other hand, Au NPs were reported to cause significant oxidative stress and cytotoxicity that could reveal a high risk potential on vital organs (Abdelhalim and Jarrar, 2011, 2012). Moreover, Au NPs are able to induce oxidative stress by interacting with cell components that could result damage to tissues, cells and macromolecules (Abdelhalim and Jarrar, 2011, 2012; Almansour and Jararr, 2017).

Silver NPs have antimicrobial activity and found in many commercial brands, such as sunscreen, medical masks, tooth paste and deodorants (Jain et al., 2009). Nanosilver accumulate mainly in the liver and considered to be toxic to other organs including kidneys and lungs, possibly through production of free radicals that damage the mitochondria and cytoskeleton (Almansour et al., 2016; Jain et al., 2009).

Zinc oxide NPs are currently being produced in various cosmetic makeup products, such as sunscreens. They have a protective effect against UV radiation and therefore can be applied in cancer prevention and therapy (Vinardell et al., 2017). However, ZnO NPs caused inflammation with damaging effect in the hepatic and renal tissues (Esmaeillou et al., 2013). In addition, ZnO NPs increased significantly the levels of serum liver enzymes, total protein, creatine kinase, and lactate dehydrogenase (Wang et al., 2010).

Copper oxide NPs can be found in antibacterial, anti-tumor and osteoporosis-treatment drugs (Fratoddi et al., 2019). These nanomaterials induce toxicological alterations, such inflammation, apoptosis

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and mitochondrial damage together with oxidative stress and lipid metabolism dysfunction, in the liver, kidney, spleen and other organs (Manna et al., 2012).

Silicon dioxide NPs have been widely used due to their fascinating physicochemical properties (He and Shi, 2014; Murugadoss et al., 2017). They are used in drug delivery, cancer treatment, and pharmaceutical additives (Barik et al., 2008; Tallury et al., 2008). Although, Al-Mansour et al., 2018, reported that SiO_2 induced histological toxicological alterations in the liver of SiO_2-exposed mice (Almansour et al., 2018).

Drug metabolism is a chemical conversion of the drugs, which usually results in inactivation of the drugs. The major site of drug metabolism is the liver, which mediates the metabolism of drugs through enzymes (Almazroo et al., 2017). These enzymes are classified into phase I and II drug-metabolizing enzymes (DME) (Almazroo et al., 2017). The major phase I DMEs are cytochrome P450 s (cyp450 s), including human CYP3A4 and CYP2C9, which metabolize more than 40 % of the total prescribed drugs (Zanger and Schwab, 2013). The major phase II DMEs are UDP- glucuronosyl transferase enzymes (UGTs), including UGT2B7 which metabolizes fatty acids and 10 % of the total prescribed drugs (Badee et al., 2019; Jarrar et al., 2014).

Foods, smoking, drugs and toxins were found to influence the hepatic metabolism of the drugs through affecting the mRNA and protein expression of the DMEs (He et al., 2015; Jarrar et al., 2019a). Regarding NPs, it is reported that Ag NPs and Au NPs inhibit the human microsomal CYP450 s using *in vitro* methods (Sereemaspun et al., 2008). Almansour et al., showed that SiO_2 NPs downregulate the mRNA expression of drug and arachidonic acid metabolizing enzymes in the liver of SiO_2-treated mice (Almansour et al., 2018). In addition, it is found that Cu NPs downregulate the mRNA and protein expression of CYP450 s in the kidney of the treated rats (Xu et al., 2018). On the other hand, it was reported that polyethylene Au NPs upregulate the mRNA expression of DME genes in HepG2 cells (Warisnoicharoen, 2011).

There is a controversial effect of different metallic NPs on the expression of DME genes. Therefore, the present study aims to find out the influence of variable widely used metallic NPs on the mRNA expression of major hepatic mouse DMEs, cyp3a11, cyp2c29 and ugt2b1, which are equivalent to human CYP3A4, CYP2C9 and UGT2B7. In addition, this study linked the alterations in gene expression of DME genes, induced by NPs exposure, with the hepatic inflammation and interleukin-6 expression, that are known to regulate the expression of DME genes (Mallick et al., 2017; Ning et al., 2017).

2. Material and methods

2.1. Nanoparticles

Five of metallic NPs (Ag NPs, Au NPs, CuO NPs, SiO2 NPs and ZnO NPs) were used in the present study. Silver NPs were obtained from the Department of Nanotechnology of Laser Zentrum-Hannover, Germany while other metallic NPs were purchased from Sigma-Aldrich (St. Louis, USA).

Gold NPs: Spherical colloidal monodisperse Au NPs (10 nm) suspended in 0.1 mM phosphate-buffered saline, were used, These NPs demonstrated the following physicochemical characterization: 5.98 × 1012 NPs/mL, concentration of 1.01 × 108 M-1 cm-1, surface area of each particle of 3.16 × 10-12 cm^2, reactant free and absorption at 520 nm.

Silver NPs: Spherical Ag NPs (15–35 nm diameter) with the maximum particle count was at 20 ± 5 nm size dissolved in deionized water containing 0.1 mM sodium citrate were used.

Zinc oxide NPs: Well-dispersed ZnO NPs, average particle size 35 nm, at 50 wt% in distilled water were used in the present study. NPs dispersion was disaggregated by ultrasonication before being diluted with sterile acidic distilled water (pH 5.5) at 37∘C immediately before use.

Silicon dioxide NPs: Spherical and porous silica powder (10 nm, purity 99.5 %, surface area of 640 m^2/g) were used. A fresh dispersion of NPs was disaggregated by ultrasonication after dilution with sterile physiological (0.9 % NaCl) saline at 37 ˚C immediately before use

Copper oxide NPs: Nanopowder of copper oxide NPs < 50 nm with an average of 20 + 5 nm, surface area of 29 m^2/g with a metal content of 99.99 % were used. A fresh dispersion of NPs was ultrasonicated after dilution in deionized water containing 0.1 mM sodium citrate were used.

All NPs solutions were prepared so that the necessary dose could be administered by intra-peritoneal route in a volume of 250 µL.

2.2. Experimental animals ant treatment

Forty-two adult male BALB/C mice (*Mus musculus*), with an average weight of 27 ± 4 g were obtained from the animal house of Jerash University. All animals were feed standard laboratory animal diet pellets *ad libitum*. The mice were treated according to Canadian Counsel of Animal Care (Rowsell, 1991). The study protocol was approved by the ethical committee at King Khalid University, Saudi Arabia. The mice were divided into control group and 6 NPs-treated groups. Each member of the control group received a daily dose of the vehicle, which is a normal physiological saline (0.9 % NaCl), while each mouse of NPs-treated groups (Au NPs treated-, CuO NPs treated-, SiO_2 NPs treated- and ZnO NPs) received a daily intra-peritoneal injection with a dose of 2 mg/kg bw of NPs for 21 days

2.3. RNA extraction and gene expression analyses

Total RNA was isolated from 200 mg of the hepatic mouse biopsies using Trizol extraction solution (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. Then, RNA was converted to complementary DNA (cDNA) by cDNA Synthesis Kit® (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. The concentration and purity of the RNA and cDNA samples were analyzed by Nanodrop instrument Quawell DNA/Protein Analyzer (Sunnyvale, CA, USA). The accepted RNA purity 260/280 ratio of RNA and cDNA was 2 ± 0.1 and 1.8 ± 0.1, respectively (Abdel-Latif and Osman, 2017).

The expression of major hepatic mouse DME genes, *cyp3a11, cyp2c29 and ugt2b1*, was analyzed using real-time polymerase chain reaction (RT-PCR), as published previously (Jarrar et al., 2018). Briefly, 1 ng of synthesized cDNA was reconstituted in a reaction mixture containing Luna® Universal Master Mix and forward and reverse primer (Integrated DNA technologies, USA) (Supplementary Table 1). The mRNA expression of tested genes was normalized through measuring the expression of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene (Livak and Schmittgen, 2001).

2.4. Histological processing

Fresh liver biopsy from each mouse under study was cut rapidly, fixed in neutral buffered formalin (10 %) and dehydrated with ascending grades of ethanol (70 %, 80 %, 90 %, 95 % and 100 %). Tissue blocks were then cleared in two changes of xylene, impregnated with two changes of molten paraffin wax (m.p. 58 C), embedded and blocked out. Sections (4–5 µm) were stained by hematoxylin and eosin stain.

2.5. Statistical analysis

The level of mRNA was expressed by the fold change in the expression of targeted gene after NP treated to the control group. One way ANOVA test was used for the statistical comparison between the groups. The difference between the groups was considered significant when the p value was less than 0.05. The statistical analysis was done using Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois) version 23 software for Windows.

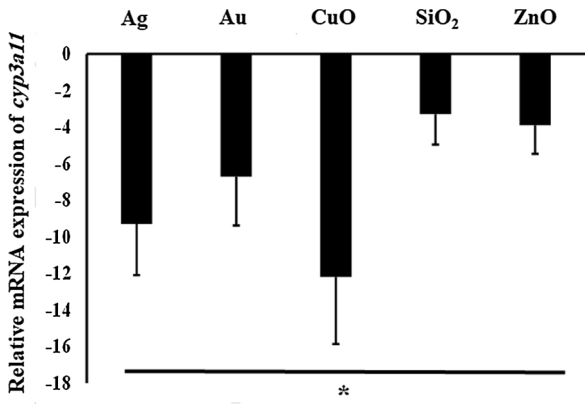

Fig. 1. The mRNA expression of mouse *cyp3a11* gene after 21 days exposure of different 5 metallic NPs. The mRNA was analyzed using real-time polymerase chain reaction technique. "*" indicates statistical difference with p value < 0.05 and using one way ANOVA test.

3. Results

3.1. The influence of metallic NPs on DME genes expression

Fig. 1 shows the relative mRNA expression of *cyp3a11* after treatment of the mice by different NPs. It is shown that all NPs caused a significant (p value < 0.05) downregulation of the expression of *cyp3a11* gene. The highest effect was observed with CuO NP, which reduced the expression of *cyp3a11* gene by more than 12 folds. The order of the influence of tested NPs on the expression of *cyp3a11* gene is: CuO > Ag > Au > SiO2 and lastly, ZnO.

Regarding the influence of NPs on the expression of *cyp2c29*, it is found that also all tested NPs reduced significantly (p value < 0.05) the mRNA levels of *cyp2c29* gene with the highest influence seen by CuO, which downregulated the expression of *cyp2c29* gene by more than 10 folds (Fig. 2).

It is found also that all tested NPs decreased significantly (p value < 0.05) the expression of *ugt2b1* gene (Fig. 3). Although the effect of CuO on the *ugt2b1* expression was higher than other tested NPs, this influence was not statistically different than other NPs.

Generally, the influence of SiO_2 and ZnO on drug-metabolizing enzymes was relatively weaker than other tested NPs, although they caused a significant downregulation in the expression of the DMEs (Figs. 1–3).

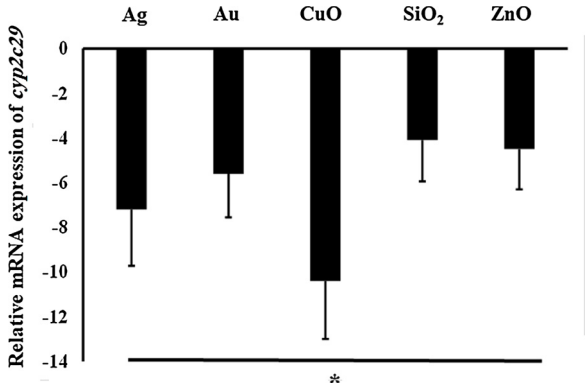

Fig. 2. The mRNA expression of mouse *cyp2c29* gene after 21 days exposure of 5 metallic NPs. The mRNA was analyzed using real-time polymerase chain reaction technique. "*" indicates statistical difference with p value < 0.05 and using one way ANOVA test.

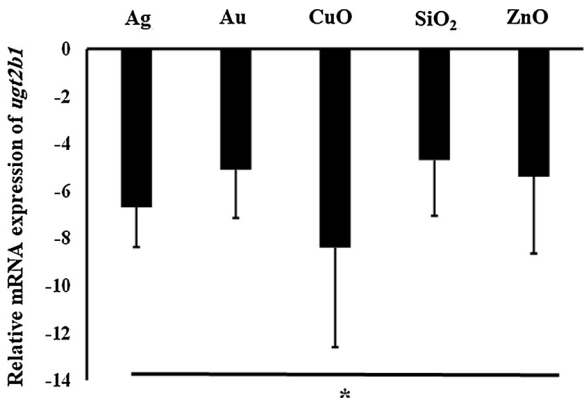

Fig. 3. The mRNA expression of mouse *ugt2b1* gene after 21 days exposure of 5 metallic NPs. The mRNA was analyzed using real-time polymerase chain reaction technique. "*" indicates statistical difference with p value < 0.05 and using one way ANOVA test.

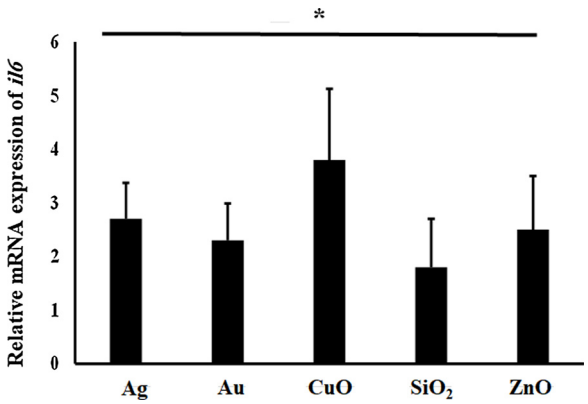

Fig. 4. The mRNA expression of mouse *il6* gene after 21 days exposure of 5 metallic NPs. The mRNA was analyzed using real-time polymerase chain reaction technique. "*" indicates statistical difference with p value < 0.05 and using one way ANOVA test.

3.2. The influence of metallic NPs on il6 gene expression

This study investigated the influence of NPs on the expression of *il6* gene, a regulator of DMEs gene expression (Wu and Lin, 2019). As it is shown on Fig. 4, all NPs upregulated significantly (p value < 0.05) the expression of *il6* gene by more 2 folds. Relatively, CuO upregulated the expression of *il6* gene by 4 folds, which is relatively higher than the influence of other treated NPs on *il6* expression.

3.3. Correlation between DMEs and il6 expression

After analyzing the mRNA gene expression, this study correlated between the expression of *il6* and the DME genes. It is found that *il6* expression is significantly (p value < 0.05) and inversely correlated with the expression of *cyp3a11*, *cyp2c29* and *ugt2b1*, as represented on Fig. 5.

3.4. Histological examination

Compared with the control livers, inflammatory cells infiltration was seen in the hepatic tissues of all tested metallic NPs-treated mice (Fig. 6a–f). This alteration was more prominent in the hepatic tissue of mice exposed to CuO NPs and Ag NPs with lesser extent of infiltration was seen the hepatic tissue of mice treated with SiO_2 NPs and ZnO NPs. Moreover, inflammatory cells infiltration was most obvious in the hepatic interstitial tissue next to the blood vessels.

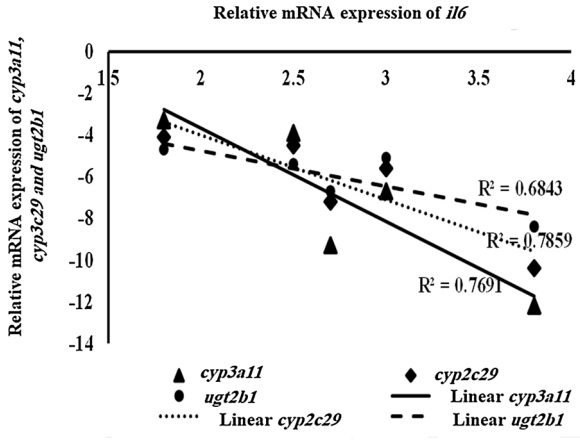

Fig. 5. Correlation between *il6* and DMEs (*cyp3a11, cyp2c29* and *ugt2b1*) gene expression. Pearson correlation analysis was used to measure the correlation (r^2) between *il6* and DMEs gene expression.

4. Discussion

NPs are well known to cause several molecular alterations in the cells among different organs (Ajdary et al., 2018). In this study, it was found that all tested NPs caused a significant downregulation of the mRNA expression of mouse *cyp3a11*, *cyp2c29* and *ugt2b1*, which metabolize most of the drugs found in the pharmaceutical market. These results may indicate that sub-chronic exposure to metallic NPs reduce the expression of DME genes, and hence affecting the pharmacokinetics and the response of the administrated drugs. This finding should be taken into consideration when administrating the drugs to patients under exposure of different metallic NPs.

Alteration in the expression of DME genes can explain, at least partly, the phenotypic changes in drug metabolism among humans. It was reported that expression of DMEs is downregulated sharply in the liver of uncontrolled diabetic mice (Jarrar et al., 2018) which is correlated with low drug-metabolism activity among human diabetic patients (Irshaid et al., 1992). In addition, the anti-epiliptic and the anti-tuberculosis rifampicin drugs upregulate the expression of DMEs, which results in reduction of the plasma drug levels and efficiency of other administrated drugs (Chen and Raymond, 2006). Since NPs downregulte the expression of DMEs, it is predicted that NPs decrease clinically the metabolism of the co-administrated drugs. This decreasing in the metabolic rate may accumulate the drugs inside the body and therefore causes drug-induced toxicity. Interestingly, it was reported that uncoated Au NPs reduce the metabolism of testosterone hormone in pooled hepatic human microsome, which might be through inhibition of human CYP450's metabolic activity (Choi and Joo, 2019).

Several studies have been shown that the expression of DMEs is downregulated in the hepatic diseases and drug induced hepatotoxicity (Elbekai et al., 2004). Non-steroidal anti-inflammatory drugs (NSAIDs) caused downregulation of hepatic drug metabolizing enzymes, which was correlated with hepatic toxicity induced by NSAIDs (Jarrar et al., 2019b). In addition, SiO_2 NPs caused hepatotoxicity to the NPs-exposed mice, which also was in parallel with downregulation of the expression of DME genes (Almansour et al., 2018). Furthermore, several studies showed that metallic NPs cause hepatotoxicity (Yao et al., 2019; Yildirimer et al., 2011). We found in the present study that all tested metallic NPs induced histological toxicological alteration in the liver of treated mice through induction of hepatic inflammation. Additionally, *il6* gene expression was upregulated after metallic NP exposure, which is a molecular marker of hepatotoxicity (Tanaka et al., 2014). These findings may indicate that hepatotoxicity induced by NPs exposure plays, at least in part, a role in the downregulation of mRNA expression of DME genes. Further studies are recommended to find out the role of hepato-protective compounds against the downregulation in drug metabolism induced by metallic NPs exposure.

It is reported by Xu et al., that Cu NP down-regulated the mRNA and protein levels of renal CYP450 s in NPs treated rats (Xu et al., 2018). In addition, Almansour et al., 2018, found that SiO_2 NPs downregulted the mRNA expression of hepatic mouse cyp450 s (Almansour et al., 2018). The results of this study are in line with the previous mentioned studies that different NPs caused a significant decline in the mRNA levels of DMEs, while our results disagree what was reported in Warisnoicharoen study, where Au NPs upregulated the expression of DME genes in HepG2 cell line after 48 h incubation (Warisnoicharoen, 2011). This disagreement in the findings might be due to different *in vitro* and *in vivo* methods used in studying the influence of NPs on the expression of DME genes.

The results of the present study showed that CuO NPs have the highest effect on the expression of *cyp3a11*, *cyp2c29* and *ugt2b1*, in comparison with other tested NPs. This variable influence on the expression of DME genes, among different tested metallic NPs, might be linked to different degree of hepatotoxicity induced by variable metallic NPs. It is reported that CuO NPs have higher toxicity than Ag NPs to the livers of *Siberian sturgeon* (Ostaszewska et al., 2016). In addition, it is found that CuO NPs were more hepatotoxic than ZnO NPs (Yahya et al., 2019). Furthermore, Ag NPs caused more hepatic harmful alterations and inhibition of microsomal human CYP450 s, than Au NPs (Sereemaspun et al., 2008; Botha et al., 2019). We found, in this study, that infiltrated cells were seen in relatively higher amounts in the liver of CuO NPs and Ag NPs treated mice, in comparison with ZnO NPs-treated group.

Some reports indicated that CuO NPs could cause hepatotoxicity through DNA breakage, mitochondrial damage, apoptosis and genotoxic through direct interacting with nuclear proteins and mitotic spindles (Magdolenova et al., 2014; Yao et al., 2019). These hepatic alterations might be resulted due to the capacity of CuO NPs to generate reactive oxidative species (ROS) (Naz et al., 2020). Interestingly, Cu is the only redox-active metal, among the tested metallic NPs under study, which was reported to induce ROS generation *via* Haber-Weiss and Fenton-type reaction mechanisms (Knaapen et al., 2004). Furthermore, CuO NPs could cause more than 2-fold downregulation of the antioxidant enzymes, such as glutathione S-transferase and superoxide dismutase, decreasing hepatocytes defense mechanism against ROS (Tuncsoy et al., 2017). A correlation was also reported between ROS production and the hepatic inflammation with or without downregulation of DME genes (Gandhi et al., 2012; Zhang et al., 2015). Accordingly, one may conclude that CuO NPs could produce more ROS, than the other NPs under study, causing more hepatic injury including induction of inflammation and downregulation of gene expression of DMEs.

Previous studies revealed significant differences between the metallic NPs regarding their accumulation in the hepatic tissues where high metallic NPs accumulation was accompanied with higher toxicity (De Jong and Borm, 2008; Colino et al., 2020). This alteration might be attributed to the observed variable influence of the used metallic NPs on the expression of DME gene expression and inflammation induction.

Interleukin-6 regulates the expression of hepatic DMEs genes (Mallick et al., 2017; Ning et al., 2017). The findings of the present work showed that all NPs under study upregulted significantly (P value < 0.05) the expression of *il6* gene. This upregulation might be resulted in increasing the protein synthesis of interleukin-6; where positive correlation between the mRNA and protein level of interleukin-6 was reported previously (Jonas et al., 2015). The mRNA level of *il6* was inversely and significantly correlated with the level of DME gene expression after exposure to different NPs. This may indicate that increased levels of *il6* gene expression downregulated the mRNA levels of DMEs. Interestingly, Xu et al., found that the levels of different interlaukins 1, 2, 4 and 6 were induced in the kidney of Cu NP-treated rats, which were correlated with downregulation of renal CYP450 s (Xu

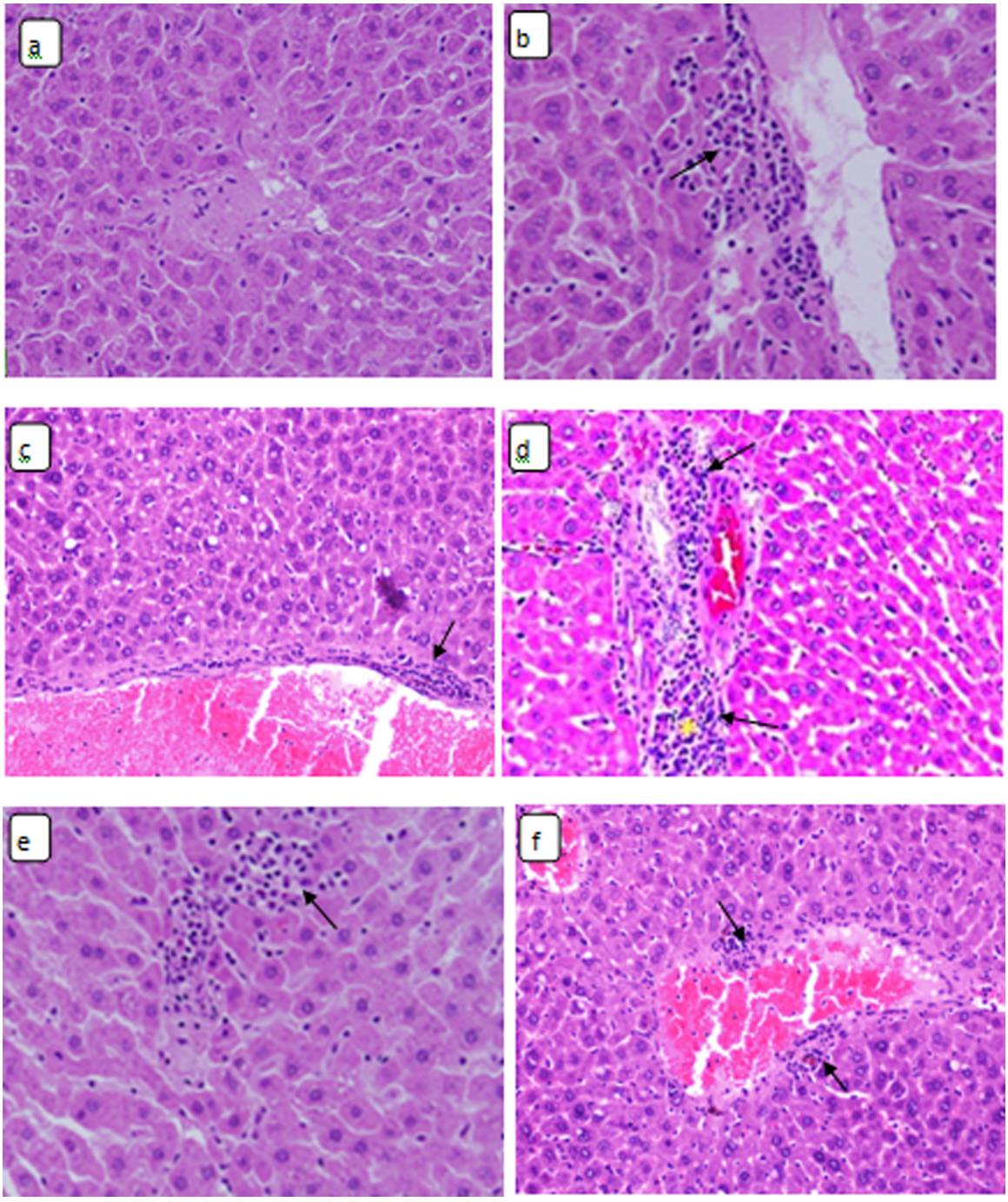

Fig. 6. (a-f). Photomicrographs of stained hepatic tissues of mice received five types of nanoparticles demonstrating inflammatory cell infiltration (arrows): (a) Ag NPs, H&E stain (b) Au NPs, H&E stain (c) CuO NPs, H&E stain (d) SiO2 NPs, H&E stain (f) ZnO NPs, H&E stain.

et al., 2018).

One of the limitations of this study is that the protein levels of DMEs were not analyzed. However, some previous studies showed an accordance between mRNA and protein levels of cyp450 s (-Zordoky et al., 2010; Jarrar et al., 2013). Furthermore, it was reported that both mRNA and protein levels of DMEs were reduced after treating the rats with Cu NPs (Xu et al., 2018). These together may indicate that protein levels of DMEs were decreased in the hepatocytes exposed to the invested metallic NPs. In addition, this may also indicate a need to find the influence of these metallic NPs on the protein expression of DMEs.

In conclusion, metallic NPs could cause a sharp downregulation in the expression of DME genes with a highest influence was seen by CuO NPs and the least with SiO_2 ones among the NPs used in the present work. In addition, the downregulation in DME genes was correlated with induction of inflammation and *il6* gene upregulation in the liver. These findings may increase our understanding on the effect of metallic NPs exposure on the drug's metabolism and the necessity of using hepato-protective agents against the toxicity induced by metallic NPs exposure.

CRediT authorship contribution statement

Yazun Jarrar: Conceptualization, Methodology, Supervision, Investigation, Validation, Data curation, Writing - original draft, Writing - review & editing. **Amin Al-Doaiss:** Conceptualization, Methodology, Supervision, Fund acquisition, Formal analysis, Data

curation, Writing - original draft, Writing - review & editing. **Mohammad Alfaifi:** Fund acquisition, Formal analysis, Data curation, Writing - original draft. **Ali Shati:** Fund acquisition, Formal analysis, Data curation, Writing - original draft. **Mohammed Al-Kahtani:** Fund acquisition, Formal analysis, Data curation, Writing - original draft. **Bashir Jarrar:** Project administration, Conceptualization, Methodology, Supervision, Investigation, Validation, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.etap.2020.103449.

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