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RESEARCH PAPER

Comparison of the effects of silver nanoparticles and silver cobalt nanoparticles on function tests and liver tissue changes in adult male rats

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ABSTRACT

Objective(s): This study aimed to compare impacts of silver nanoparticles and silver cobalt nanoparticles on the hepatic function tests and changes in liver tissues in adult male rats.

Materials and Methods: This study was conducted on 49 adult male Wistar rats, each weighing approximately 180-220 gr. The rats were randomly assigned to seven groups of seven including one control group and six experimental groups. The experimental groups 1 and 2 respectively received 25 and 100 mg/kg of silver nanoparticles synthesized for 75 sec intraperitoneally for 14 days. Experimental group 3 received silver nanoparticles that were synthesized at 300 sec which were administered intraperitoneally in a 25 mg/kg dose for 14 days. The experimental groups 4 and 5 received silver cobalt nanoparticles, whereby silver nanoparticles were synthesized at 75 sec and were administered intraperitoneally in a 25 and 100 mg/kg dose for 14 days, respectively. Finally, experimental group 6 received a 25 mg/kg dose of silver cobalt nanoparticles, intraperitoneally for 14 days, with the silver nanoparticles synthesized for 300 sec. At the end of this period, the serum levels of hepatic enzymes, albumin, and total protein were measured and tissue changes were evaluated in this study.

Results: The mean serum levels of AST, total protein, and albumin in the experimental groups 1 and 3 increased significantly compared to the control group. Similarly, the mean serum levels of ALT and ALP in the experimental group 3 showed a significant increase in comparison with the control group. The mean of liver weight in all experimental groups was significantly higher than the control group($P<0.05$). Furthermore, the necrosis of the liver tissue was observed in the recipients of silver nanoparticles. However, liver necrosis was not observed in the groups receiving silver cobalt nanoparticles.

Conclusion: The use of silver nanoparticles can boost the serum levels of hepatic enzymes and increase liver tissue necrosis, as well. However, the silver cobalt nanoparticles did not change the levels of hepatic enzymes and liver tissue.

Keywords: *Albumin, Hepatic Enzyme, Rat, Silver Nanoparticles, Silver Cobalt Nanoparticles*

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INTRODUCTION

There is a growing increase of the application of nanotechnology products in human activity. Silver nanoparticles (Ag NPs) are nanoparticles of silver with the size of 1-100 nanometer [1]. The effects of silver nanoparticle poisoning on none-mammalian species, cell lining, and bacteria have been proven in the literature [2]. Studies of in vitro have shown that poisoning with silver nanoparticles includes reactive oxygen species production with oxidative stress, interaction with enzymes and proteins to free thiol group, and the mimic of endogenous ions of calcium, sodium, and potassium. These mechanisms result in cytokine production, cellular damage, and ultimately cell death and necrosis.

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The in vitro studies reported that the effects of cell and genetic toxicity of the silver nanoparticle are related to the dose, size, type of cell and its coating [3-5].

Kim et al. (2009) demonstrated that human liver cell poisoning with silver nanoparticles depends on oxidative stress. In particular, the authors reported that treatment with silver nanoparticles induced superoxide dismutase levels. Their findings showed that the primary toxicity of the silver nanoparticle was due to oxidative stress, rather than the effects of silver ions [6]. More recently, Lim et al. (2017) showed that silver nanoparticles possess anti-cancerous properties in human cancer cells that modulate these effects by DNA-dependent protein kinase [7]. Guo et al. (2017) similarly that exposure to silver nanoparticles *in utero* or in early infancy may lead to developmental abnormalities [8].Using a rat model, Gooschian et al. (2017) demonstrated that silver nanoparticle increase the expression of the Bax genes, while reducing the expression of Bcl-2 genes. As a result, the Bax/Bcl-2 ratio increases in rat hippocampal cells. The authors further noted that these gene expression changes induced cell death, resulting in nerve poisoning by silver nanoparticle [9]. Similarly, Liu et al. (2017) demonstrated that silver nanoparticles have a negative effect on spatial perceptions and the formation of synaptic hypocompatibillities. Specifically, it was found that silver nanoparticles, in both low and high doses, lead to abnormal long-term potentials [10].

In a study by Wan et al. (2017), it was demonstrated that cobalt nanoparticle results in pulmonary damage, damage to DNA and cell proliferation. The authors attributed these changes to oxidative stress and inflammation of the lung [11]. Similarly, Liu and Han (2017) determined that low-dose nano-cobalt induces cell death and regulates osteogenic differentiation in the MG-63cells [12]. More recently, Xu et al. (2018) showed cobalt nanoparticles and ions induce macrophage retention [13].

In addition, Liu et al. (2017) determined that cobalt and cobal nanoparticles increase the volume of reactive oxygen species and inflammatory cytokines, including tumor necrosis factor alpha, interleukin 1 beta, and interleukin 6, which leads to cellular poisoning [14].

Since silver and silver cobalt nanoparticles can have detrimental side effects when used for therapeutic purposes, such as induction of oxidative stresses and increased free radicals production, and given that liver is a vital organ, the aim of the present study was to investigate the possible influence of different doses of silver nanoparticles and silver cobalt nanoparticles on hepatic enzyme levels (AST, ALT, ALP), albumin and total protein, as well as histological changes in the liver of male rats. The examination of potential widespread actions of these particles may lead to the generation of novel therapies and provide fresh insight into the therapeutic use of these agents in the treatment of complex disorders.

MATERIALS AND METHODS

Synthesis of silver nanoparticles and silver cobalt nanoparticles

The Ag NPs were synthesized by Controlled Current Coulometry (CCC), which is a widely utilized and electrochemical method. A solution mixture of $AgNO_3$ (Silver nitrate) and KNO3 (Potassium nitrate) was applied as an electrolyte, while PVP (Polyvinylpyrrolidone) served as a stabilizer. In Ag NPs preparation, different time intervals of electrolysis were used (75 sec, and 300 sec), at room, while keeping the cathode electrode rotating speed at 3000 rpm and the current at 1 A. The color change of the electrolyte around the cathode to yellow revealed the formation of Ag NPs. The Ag/Co core-shell NPs were prepared by the reduction method, using Ag NPs as a core. For this purpose, a solution containing C_oSO_4 (Cobalt sulfate) and CTAB (Cetrimonium bromide or Cetyltrimethylammonium bromide) was added to the colloidal Ag NPs solution. Next, $NaBH_4$ (Sodium borohydride) was added as a reducing agent. The color change to dark brown indicated the formation of Ag/Co NPs [15].

Animals

All the procedures employed in this experimental study were approved by the Institutional Animal Care and Ethical Committee of Shiraz Islamic Azad University. The sample comprised of 2.5- to 3-month-old 49 adult male Wistar rats 180-220 gr. They were randomly assigned to groups of 7, all of which were kept in standard cages at 22-20 °C and were subjected to 12 h light and 12 h dark cycles. All the groups had free access to food and water.

Animal treatment

The aforementioned seven groups of seven

rats each comprised of one control group and six experimental groups. The experimental groups 1 and 2 respectively received 25 and 100 mg/kg of silver nanoparticles synthesized for 75 sec intraperitoneally for 14 days. Experimental group 3 received silver nanoparticles that were synthesized at 300 sec, which were administered intraperitoneally in a 25 mg/kg dose for 14 days. On the other hand, the experimental groups 4 and 5 received silver cobalt nanoparticles, whereby silver nanoparticles were synthesized at 75 secand were administered intraperitoneally in a 25 and 100 mg/kg dose for 14 days, respectively. Finally, experimental group 6 received a 25 mg/kg dose of silver cobalt nanoparticles, intraperitoneally for 14 days, with the silver nanoparticles synthesized for 300 sec. Intake doses, injection type, and duration of injection were selected in line with the procedures adopted in previous studies [16-18].

At the end of the treatment period, animals were anesthetized by ether, the liver weight of the rats was measured by digital scales, and blood samples were collected from the left ventricle of the heart. The blood samples were kept in the laboratory for 20 min, and centrifuged for 15 min at 5000 rpm. Next, the serum of each sample was collected and used for the measurement of hepatic enzymes and protein level. The AST and ALT levels were measured by DGKC phosphate buffer, and ALP level was measured by P-Nitrophenyl phosphate AMP method. The biuret reaction endpoint method was used to measure total protein. The Bromocresol Green method was used to measure albumin [19 and 20].

Histological experiments

The liver was removed after necropsy. The tissues were stabilized in formalin buffer 10%. The dehydration was accomplished by the application of alcohol with different concentrations (from low to high).

For clearing, the tissues were placed in two Xylene containers. In the infiltration stage, the tissues were soaked in melted paraffin (65 °C), each for 1 hr. Leukhardt parts were used in the molding stage. All tissue slides were cut at 4-5 micron and hematoxylin-eosin was used to stain the tissues. All histological evaluations were performed under the supervision of an experienced pathologist [21].

Statistical analysis

The SPSS software (version 22, Chicago, IL, USA) was used for data analysis. The ANOVA test was performed on the data. Duncan test was used to evaluate the significant differences in the data. P-value less than 0.05 were considered statistically significant. The serum levels of ALT, AST, ALP, albumin, and total protein were presented as Mean ± SEM.

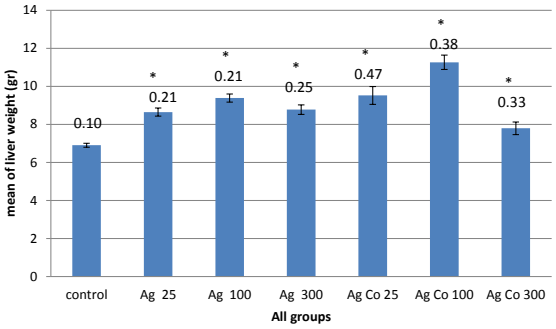

Fig 1. Comparison of mean liver weight between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles and the control group
*There was a significant difference between the experimental groups receiving silver nanoparticles and silver cobalt nanoparticles with the control group (P<0.05). Values were reported based on the Mean ± SEM

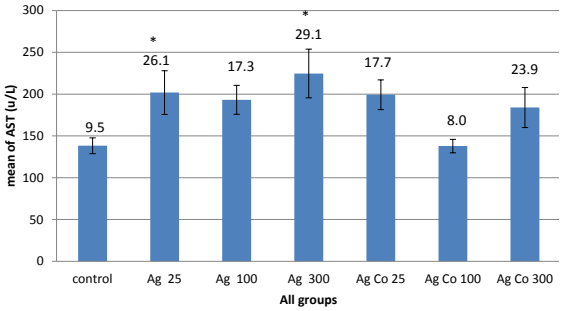

Fig 2. Comparison of mean concentration of AST enzyme between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles with the control group.
*There was a significant difference between the experimental groups receiving silver nanoparticles with the control group at the level of P<0.05. Values were based on the Mean ± SEM

RESULTS

The statistical analysis and mean serum levels of AST, ALT, ALP, albumin, and total protein were performed in the experimental groups receiving silver nanoparticles and silver cobalt nanoparticles. The obtained results of the experimental group were compared with those of the control group. The mean weight of the liver in all experimental groups receiving silver nanoparticles showed a significant increase compared to the control group. Furthermore, the mean liver weight in

all experimental groups receiving silver cobalt nanoparticles showed a significant increase compared to the control group (P<0.05; Fig 1).

The mean serum levels of AST enzyme in the experimental groups 1 and 3 receiving silver nanoparticles were significantly higher than the control group. However, the serum level of total protein in the experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles did not significantly change, compared to that of the control group (P<0.05; Fig 2).

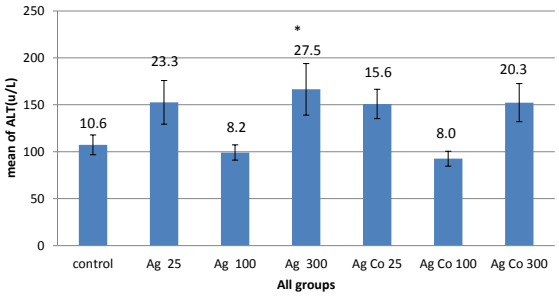

Fig 3. Comparison of mean concentrations of ALT enzyme between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles with the control group.
*There was a significant difference between the experimental groups receiving silver nanoparticles with the control group at the level of P<0.05. Values were based on the Mean ± SEM

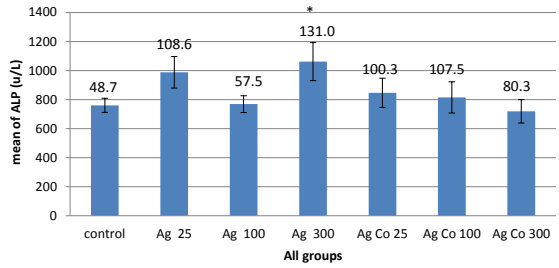

Fig 4. Comparison of the mean concentrations of ALP enzyme between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles and the control group
*There was a significant difference between the experimental groups receiving silver nanoparticles and the control group (P<0.05). Values were based on the Mean ± SEM

Similarly, the mean serum levels of ALT enzyme in the experimental group 3 receiving silver nanoparticles increased significantly, compared to that of the control group. However, the serum level of ALT enzyme in the experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles did not significantly change compared to that of the control group (P<0.05; Fig 3).

The mean serum level of ALP enzyme in the experimental group 3 receiving silver nanoparticles was significantly higher than that of the control group. However, the serum level of ALP enzyme in the experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles did not significantly change compared to that of the control group (P<0.05; Fig 4).

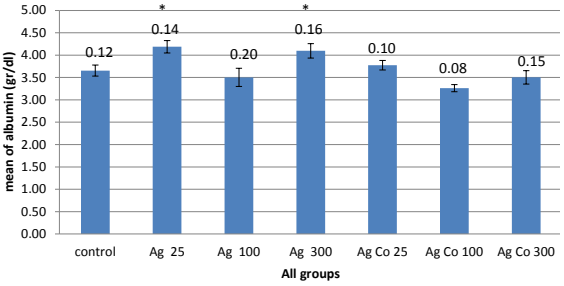

Fig 5. Comparison of mean concentrations of albumin between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles and the control group
*There was a significant difference between the experimental groups receiving silver nanoparticles and the control group (P<0.05). Values were based on the Mean ± SEM

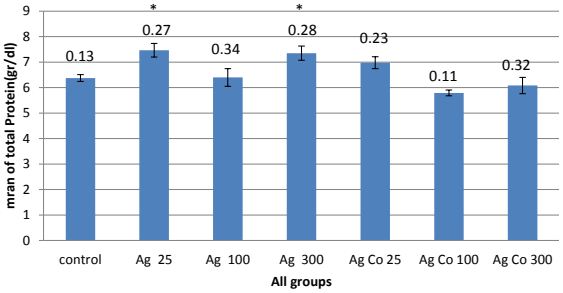

Fig 6. Comparison of mean concentrations of total protein between experimental groups receiving silver nanoparticles and silver cobalt nanoparticles and the control group
*There was a significant difference between the experimental groups receiving silver nanoparticles with the control group (P<0.05). Values were reported based on the Mean ± SEM

Moreover, the mean serum levels of albumin in the experimental groups 1 and 3 receiving silver nanoparticles were significantly higher than that of the control group. However, the serum level of albumin in the experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles did not significantly change compared to the control group (P<0.05; Fig 5).

The mean serum levels of total protein in the experimental groups 1 and 3 receiving silver nanoparticles was significantly higher than that of the control group. However, the serum level of total protein in the experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles did not significantly change compared to that of the control group (P<0.05; Fig 6).

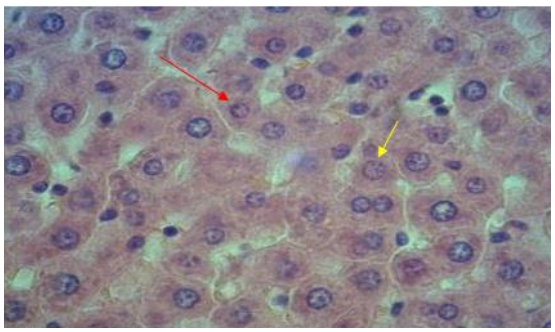

Fig 7. Photomicrograph of liver tissue of the rats in the control group. In this group, the liver tissue seemed completely normal and did not have cell damage (red arrow). All hepatocytes were healthy (yellow arrow Hematoxylin-eosin staining, magnification 40×)

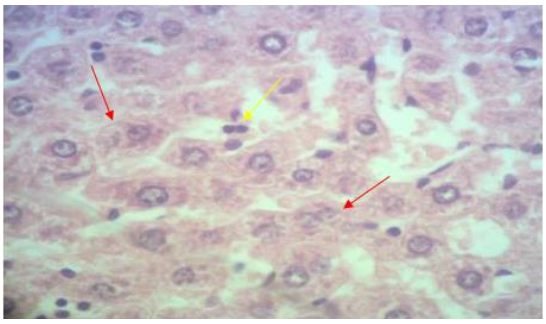

Fig 9. Photomicrograph of liver tissue of the rats in the experimental group 2 receiving 100 mg/kg of silver nanoparticles and were synthesized at 75 seconds interval. In this group, necrosis was more intense (red arrow), and there were also signs of hemorrhage (yellow arrow Hematoxylin-Eosin staining, magnification 40 ×)

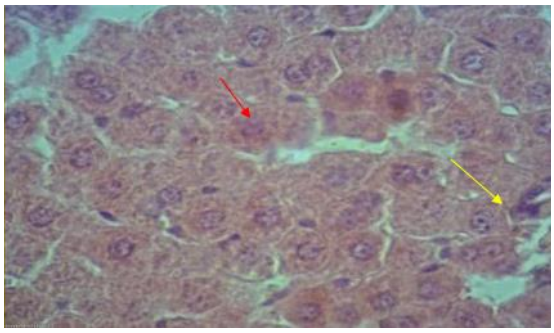

Fig 8. Photomicrograph of liver tissue of the rats in the experimental group 1 receiving 25 mg/kg of silver nanoparticles and were synthesized at 75 seconds interval. There were slight changes in this group compared to the control group. In this group, necrosis (red arrow) and cellular congestion were observed (yellow arrow Hematoxylin-Eosin staining, magnification 40 ×)

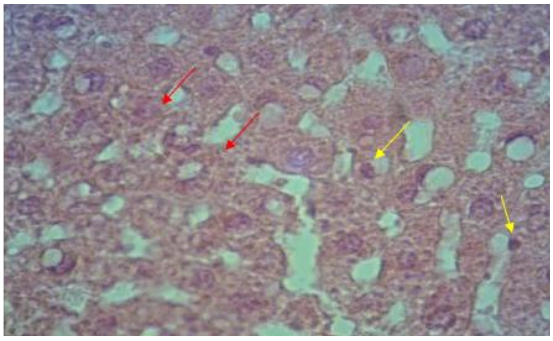

Fig 10. Photomicrograph of liver tissue of the rats in the experimental group 3 receiving 25 mg/kg of silver nanoparticles and were synthesized at 300 seconds interval. In this group, more severe cell damage was observed compared to other experimental groups (red arrow). Excessive hemorrhage, necrosis, and liver cell congestion were observed (yellow arrow Hematoxylin-Eosin staining, magnification 40×)

Histological findings

The histological analysis of the results showed that the liver tissues were completely normal in the control group with no cell damage. They had normal cellular order and maintained the radial and natural state observed in normal livers (hepatocytes have one nucleus or two nuclei), and the presence of nucleolus and central venous were two features (Fig 7).

However, in the experimental group 1, there was a slight histological variation compared to the control group, and liver tissues changed slightly. In addition, there was an observation of hepatic necrosis and cellular congestion.

In comparison with the experimental group 1, experimental group 2 showed more damage. Cellular damaging was seen as swelling, cytoplasmic swelling, nuclear swelling, vacuole formation, hemorrhage, and necrosis (Figs 8 and 9).

Moreover, the obtained results of experimental group 3 showed more cell damage than experimental groups 1 and 2.

As mentioned, cellular damaging was seen as swelling of the cytoplasm, nuclear swelling, vacuole formation, abnormal hemorrhage, cellular congestion, and necrosis (Fig 10).

The liver tissue wasin experimental groups 4, 5, and 6 receiving silver cobalt nanoparticles without cell damage.

In these experimental groups, the liver tissue had a regular cellular structure.

Normal liver cells and the presence of the nucleus and central venous were their specific characteristics (Figs 11, 12, and 13).

Excessive hemorrhage, necrosis, and liver cell congestion were observed (yellow arrow Hematoxylin-Eosin staining, magnification 40×).

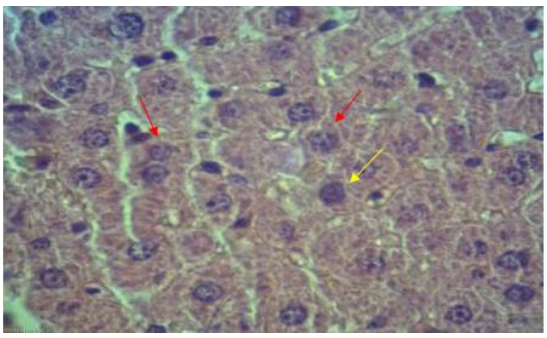

Fig 11. Photomicrograph of liver tissue of the rats in the experimental group 4 received 25 mg/kg of silver cobalt nanoparticles with silver nanoparticles synthesized at 75 seconds interval. In this group, the liver tissue looked completely normal and did not have cell damage (red arrow). No bleeding was seen in this group and no single-core infiltration was observed in hepatocytes (yellow arrow Hematoxylin-eosin staining, magnification 40×)

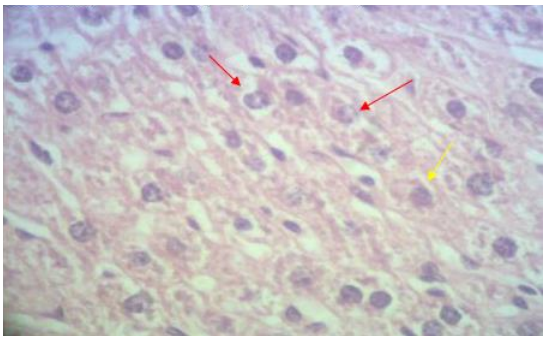

Fig 12. Photomicrograph of liver tissue of the rats in the experimental group 5 receiving 100mg/kg of silver cobalt nanoparticles, with silver nanoparticles synthesized at 75 seconds interval. In this group, the hepatic tissues appeared completely normal and did not show cellular damaging (red arrow). All hepatocytes were healthy. Necrotic cells were not seen in the liver tissue (yellow arrow Hematoxylin-eosin staining, magnification 40×)

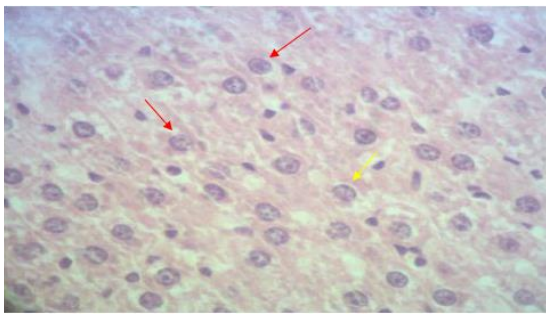

Fig 13. Photomicrograph of liver tissue of the rats in the experimental group 6 receiving 25mg/kg of silver cobalt nanoparticles, with silver nanoparticles synthesized at 300 seconds interval. In this group, the hepatic tissues appear completely normal and did not show cellular damaging (red arrow). All hepatocytes were healthy. The symptoms of hemorrhage and necrosis were not observed in this group (yellow arrow Hematoxylin-eosin staining, magnification 40×)

DISCUSSION

According to the results, the serum levels of aspartate aminotransferase enzyme, albumin, and total protein in the experimental groups 1 and 3 receiving silver nanoparticles showed a significant increase compared to the control group. Likewise, mean serum alanine aminotransferase enzyme and alkaline phosphatase level in experimental group 3 receiving silver nanoparticles showed a significant increase compared to the control group. Furthermore, necrosis was observed in the experimental groups receiving silver nanoparticles.

In a study by Li et al. (2014), it was shown that silver nanoparticles can react with the bone marrow and the liver of the rats, which may cause cellular toxicity of reticulocytes and oxidative DNA damage [22]. Silver nanoparticles can penetrate inactively into cellular organs, including mitochondria, which impairs the membrane potential and induces the production of reactive oxygen species (ROS). Oxidative stress increases with the accumulation of ROS, followed by cellular poisoning with the silver nanoparticle [23]. The utilization of in in a study was indicative of silver nanoparticle-induced liver toxicity by reducing ATP and the reduction of the activity level of antioxidant enzymes, especially glutathione [24].

Moreover, in a study by Paio et al. (2011), it was shown that silver nanoparticle-induced oxidative stress damage in the human liver by inhibiting glutathione reduction and inducing mitochondrial-dependent cell death. Additionally, silver nanoparticles induced mitochondrial-dependent cell death by modulating the expression of Bax and BCL-2 genes, which resulted in impairment of mitochondrial membrane potential [25].

In a study conducted by Xue et al. (2016), it was indicated that silver nanoparticle-induced cell death and cellular poisoning in human HepG2 liver cells. Exposure to silver nanoparticle may result in a disturbance in the G2/M phase, significant increase of the planned cell death, and generation of reactive oxygen (ROS) and matrix metalloproteinase (MMP) [26].

Regarding the effects of silver nanoparticles on liver function tests and liver tissue changes, it seems that the obtained results of this study were consistent with the results of the above-mentioned studies. This means that silver nanoparticles, through the formation of oxidative stress and reactive oxygen species (ROS), damage the liver tissue and increase the levels of liver enzymes and biochemical factors associated with liver in rats.

According to the results of the present study, the mean levels of liver enzymes (AST, ALT, and ALP), albumin and total protein in the experimental groups 4, 5, and 6 receiving the silver cobalt nanoparticle did not change significantly compared to the control group. In the experimental groups 4, 5, and 6 receiving the silver cobalt nanoparticle, the liver tissue was healthy and unchanged compared to the control group.

The central core of silver cobalt nanoparticle is composed of the silver nanoparticle. Shiva stave et al. (2016) found that the exposure of mice to silver nanoparticles induced oxidative stress. Exposure to silver nanoparticles increased the signs of inflammation, interleukin6, and nitric oxide synthase, which indicated liver toxicity [27]. In a study performed by Faed maleki et al. (2016), the silver nanoparticle reduced the content of ATP in cultured mouse cells, resulting in mitochondrial damage, and generated reactive oxygen species in dose-dependent designs. Silver nanoparticles can reduce the ability of cellular life [28].

Moreover, Heydarnjad et al. (2015) demonstrated that exposure to silver nanoparticles increased the levels of liver enzymes (AST and ALT) and liver damage in the treated mice compared to control group [29]. In a study conducted by Reshi et al. (2017), it was revealed that silver nanoparticles have protective effects on acetaminophen-induced liver toxicity. Animals treated with silver nanoparticles showed a decrease in lipid peroxidation and the reduced glutathione in their liver was corrected. Silver nanoparticles also improved the levels of liver enzymes (AST, ALT, and LDH) and bilirubin in dose-dependent designs [30].

The silver cobalt nanoparticle coating is composed of cobalt nanoparticle. One study reported that exposure to cobalt ferrite nanoparticles resulted in the increased expression of genes associated with oxidative stress, planned cell, damaged DNA, and cell damage [31]. The histological findings proved that exposure to cobalt nanoparticles could lead to more liver damage compared to cadmium chloride. Cobalt accumulates in the liver, kidneys, pancreas, and heart. Salts and minerals cobalt induced oxidative stress through reactive oxygen species and inhibited DNA repair [32].

Studies have shown that exposure to cobalt chloride leads to liver toxicity in rats [33]. In a study by Liu et al. (2016), it was shown that nanoparticles of cobalt induced more genetic and cellular toxicity than cobalt in BRL-3A cells. In this study, it was determined that cell membrane damage, oxidative stress, immune inflammation, and DNA damage may play a role in the effects of cobalt nanoparticles on liver cells [34].

Abudayyak et al. (2017) also showed that the cobalt ferrite nanoparticle has cell death effects on HepG2 and caco2 cells in concentration-dependent designs and the effects of necrotic on SH-SY5Y and A549. Cobalt ferrite nanoparticle induces damage to DNA and oxidative damage, increases the level of malondialdehyde and reduces glutathione [35]. Considering the effects of silver cobalt nanoparticles on function tests and liver tissue changes, it seems that the obtained results of this study were not consistent with the findings of other researchers. It seems that in this study, silver cobalt nanoparticles did not cause liver toxicity due to low dose and short duration of the test.

CONCLUSION

In general, the results of the present study showed that silver nanoparticles have adverse effects on liver function tests and hepatic tissue in adult male rats. However, the silver cobalt nanoparticles have no undesirable effects on function tests and liver tissue in adult male rats.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this manuscript.

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