Protective Effects of Gallic acid and Curcumin on Serum Levels of Hepatic Transaminases, Blood Plasma Parameters and Pituitary-testicular Hormones in Rats Treated Nickel Nanoparticles

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ABSTRAK

Khezri Motlagh R, Vahdati A, Hosseini SE, Edalatmanesh MA. 2021. Efek protektif asam galat (GA) dan Kurkumin (Cur) pada transaminase hati, parameter plasma darah dan kadar hormon hipofisis-testis pada tikus yang diberi NiNPs. JITV 27(1):45-56. DOI:http://dx.doi.org/10.14334/jitv.v27i1.2971

Nanopartikel nikel (NiNPs) memiliki efek toksik pada sel-sel tubuh karena memproduksi radikal bebas. Tujuan dari penelitian ini adalah untuk mengetahui efek protektif asam galat (GA) dan Kurkumin (Cur) pada transaminase hati, parameter plasma darah dan kadar hormon hipofisis-testis pada tikus yang diberi NiNPs. Tujuh puluh ekor tikus wistar jantan dewasa dibagi dalam 7 kelompok yang terdiri dari 10 ekor yaitu kontrol, Ni50 mg/kg, Ni50+GA150 mg/kg, Ni50+GA300 mg/kg, Ni50+Cur150 mg/kg, Ni50+Cur300 mg/kg dan Ni50+ GA300+CUR300 mg/kg. NiNPs, GA dan Cur diberikan secara oral dengan *gavage* oral selama 28 hari. Pada penelitian tahap terakhir, sampel darah diambil langsung dari jantung dan kadar serum transaminase hati (alanine aminotransferase (ALT) dan aspartate aminotransferase (AST)), parameter plasma darah (Glukosa, protein total (TP), bilirubin (Bil), albumin (Alb), kreatinin (Cr), Blood urea nitrogen (BUN), trigliserida, kolesterol, HDL, LDL dan alkaline phosphatase (ALP)) dan hormon pituitari-testis (FSH, LH, testosteron dan dihidrotestosteron) dinilai. Pemberian NiNPs meningkatkan kadar glukosa serum, ALT, ALP, AST, Bil, BUN, Cr, trigliserida, kolesterol dan LDL dibandingkan dengan kelompok kontrol ($p<0,05$) dan sebaliknya menurunkan kadar serum FSH, LH, testosteron, dihidrotestosteron, Alb, TP dan HDL ($p<0,05$). Namun, pemberian bersama GA dan Cur pada dosis 300 ml/kg pada tikus yang diobati dengan NiNPs meningkatkan semua parameter plasma darah dibandingkan dengan kelompok kontrol ($p>0,05$). Temuan penelitian ini menunjukkan bahwa pemberian bersama GA dan Cur pada dosis 300 mg/kg dapat mengurangi dan meningkatkan efek merusak NiNPs pada parameter plasma darah, transaminase hati dan hormon pituitari-testis pada tikus dewasa.

Kata Kunci: Nickel Nanoparticles, Curcumin, Gallic Acid, Testosterone, Hepatic Transaminases

ABSTRACT

Khezri Motlagh R, Vahdati A, Hosseini SE, Edalatmanesh MA. 2021. Protective Effects of Gallic acid and Curcumin on Serum Levels of Hepatic Transaminases, Blood Plasma Parameters and Pituitary-testicular Hormones in Rats Treated with Nickel Nanoparticles. JITV 27(1):45-56. DOI:http://dx.doi.org/10.14334/jitv.v27i1.2971

Nickel nanoparticles (NiNPs) have toxic effects on body cells due to the production of free radicals. The purpose of this research was to investigate the protective effects of Gallic acid (GA) and Curcumin (Cur) on hepatic transaminases, blood plasma parameters and pituitary-testicular hormones levels in NiNPs-treated rats. Seventy adult male Wistar rats were divided in 7 groups of 10 including control, Ni50 mg/kg, Ni50+GA150 mg/kg, Ni50+GA300 mg/kg, Ni50+Cur150 mg/kg, Ni50+Cur300 mg/kg and Ni50+GA300+CUR300 mg/kg. NiNPs, GA and Cur were administered orally by oral gavage for 28 days. At the last phase of the study, the samples of blood were taken directly from heart and serum levels of hepatic transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), blood plasma parameters (Glucose, total protein (TP), bilirubin (Bil), albumin (Alb), creatinine (Cr), Blood urea nitrogen (BUN), triglyceride, cholesterol, HDL, LDL and alkaline phosphatase (ALP)) and pituitary-testicular hormones (FSH, LH, testosterone and dihydrotestosterone) were assessed. NiNPs administration increased serum levels of glucose, ALT, ALP, AST, Bil, BUN, Cr, triglyceride, cholesterol and LDL compared to the control group ($p<0.05$) and in contrast, it decreased serum levels of FSH, LH, testosterone, dihydrotestosterone, Alb, TP and HDL ($p<0.05$). However, co-administration of GA and Cur at doses of 300 ml/kg in NiNPs -treated rats improved all blood plasma parameters compared to the control group ($p>0.05$). The findings of this study suggest that co-administration of GA and Cur at a dose of 300 mg/kg can reduce and improve the damaging effects of NiNPs on blood plasma parameters, hepatic transaminases and pituitary-testicular hormones in adult rats.

Key Words: Curcumin, Gallic Acid, Hepatic Transaminases, Nickel Nanoparticles, Testosterone

INTRODUCTION

The application of nanoparticles has increased significantly in recent years in domestic and industrial processes. These particles show special physical and chemical behavior due to their high surface-to-volume ratio and small size, therefore, they can penetrate small molecules (such as water, oxygen, and carbon dioxide) and cause rupture in their structure. Metal nanoparticles have wide applications in medicine and industry as catalysts, pigments and sensors (Abudayyak et al. 2020). Nickel nanoparticles (NiNPs) are among those with the highest frequency of use in metal nanoparticles group, therefore, the probability of exposure to NiNPs has greatly increased.

Some studies suggest that NiNPs can induce apoptosis, oxidative stress, and DNA damage (Hu et al. 2020). The accumulation of metal ions in cells increases the production of reactive oxygen species (ROS). If ROS is not neutralized by the body's antioxidant system, it can lead to oxidative stress. Oxidative stress caused by excessive ROS can lead to lipid peroxidation, DNA and protein degradation. Exposure to compounds and nickel ions in different environments can occur through skin contact, gastrointestinal tract, and inhalation of airborne particles (Marzban et al. 2020). Studies have indicated that exposure to nickel or its compounds has the potential to cause a variety of histopathological effects such as skin inflammation and also swelling, redness, eczema and itching on the skin and may also include allergic and teratogenic reactions. The International Agency for Research on Cancer has classified nickel compounds as carcinogens (Kong et al. 2014). In addition, NiNPs can induce liver, pulmonary, renal and reproductive toxicity (Abudayyak et al. 2020; Kong et al. 2014). Nickel ions can induce a wide range of adverse effects on reproduction and growth, such as influencing infertility or fertility in males and females, abortion, and congenital anomalies and defects. Hormonal disorders may play an important role in the reproductive toxicology of NiNPs at neuroendocrine and gonadal levels along the hypothalamic-pituitary-gonadal axis (Forgacs et al. 2012; Kong et al. 2014).

Today, researchers have focused on dietary supplements and natural extracts of various plants to control the toxicity of drugs and chemicals. Studies suggest that phenolic compounds have the ability to neutralize free radicals due to their antioxidant activity. The antioxidant activity of phenolic agents is mainly due to their redox properties, which allows them to act as hydrogen donors and regenerative intermediaries (Moradi et al. 2021). Gallic acid (GA), 3,4,5-trihydroxybenzoic acid, is a polyphenolic compound found in fruits, vegetables and herbal medicines. Biological and pharmacological activities of GA include antioxidant, antimicrobial, anti-cancer, anti-inflammatory and inhibitory activities of metabolic disorders (Yang et al. 2020). Animal studies suggest that oral administration of GA in diabetic rats may be effective in reducing hyperglycemia due to its antioxidant properties. Gallic acid can also significantly reduce oxidative stress by strengthening the body's natural antioxidant system against ROS. Although GA is believed to have low side effects and no serious side effects have been reported, doses greater than 1 mg/kg may have teratogenic effects to the fetus (Hsieh et al. 2015). Therefore, choosing the appropriate dose of GA can be a challenge in studies (Rahimifard et al. 2020; Variya et al. 2019). Gallic acid destroys free radicals by giving them hydrogen. In diclofenac-treated rats, the administration of GA has been shown to reduce diclofenac-induced renal toxicity by modulating oxidative stress and inhibiting inflammation (Moradi et al. 2021). Also, the administration of GA can have protective effects on liver and testicular tissue (Kahkeshani et al. 2019).

Curcumin (Cur, C21H20O6) is a polyphenolic and lipophilic compound. Curcumin as the main active curcuminoid pigment is obtained from turmeric rhizomes and does not dissolve in acid or neutral water but is soluble in acetone, methanol and ethanol. Curcumin has anti-inflammatory, antioxidant and anti-mutagenic activities due to its unique chemical structure as well as the presence of hydroxyl and methoxy groups in its structure (Jakubczyk et al. 2020). In addition, Curcumin can inhibit the proliferation of inflammatory cells due to its interaction with various molecular targets and acts as a chemical inhibitor. These properties are associated with the regulation of proinflammatory cytokines, Nitric oxide synthase enzymes (iNOS), Cyclooxygenase-2 (COX-2), Lipoxygenase, and malondialdehyde reduction. It has been suggested that Curcumin can exert its anti-inflammatory, anti-tumor, and antioxidant effects by modulating various cellular signaling pathways. The anti-inflammatory mechanism of Cur is associated with nuclear factor kappa β (NF-κB) inhibition, which leads to inhibiting the expression of proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α (TNF-α) (Coelho et al. 2020; Jakubczyk et al. 2020). Also, research shows that Cur as an effective antioxidant may reduce the effects of oxidative stress due to its ability to interact with various molecular mechanisms. This property of Cur is related to its ability to chelate heavy metals or regulate the activity of many enzymes (Alizadeh & Kheirouri 2019). The US Food and Drug Administration (FDA) has approved Cur as a safe compound based on animal and human studies. Specific mutagenicity and genotoxicity following Cur use has not been reported, even at high doses. However, due to its anti-proliferative activity, Cur may reduce cell life in

normal cells (Soleimani et al. 2018; Alizadeh & Kheirouri 2019). The positive effects of Cur on lowering serum levels of glucose, triglycerides, cholesterol and LDL and increasing serum HDL levels have been represented in some studies (Marton et al. 2021). It has been suggested that Cur may increase sperm chromatin quality and prevent the disruption of pituitary-testicular hormones (Shahedi et al. 2021; Onwuemene et al. 2019).

Due to this fact that few studies have been performed on the effect of NiNPs on hepatotoxicity and pituitary-testicular hormones and also, information on the protective effects of applying GA and Cur alone and in combination following the administration of NiNPs in male rats is insufficient, therefore, this study was designed to evaluate the administration of GA and Cur alone and in combination on changes in hepatic transaminases including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), some blood plasma parameters including glucose, total protein (TP), bilirubin (Bil), albumin (Alb), creatinine (Cr), blood urea nitrogen (BUN), triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and alkaline phosphatase (ALP) as well as pituitary-testicular axis hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and dihydrotestosterone in adult rats treated with NiNPs to provide grounds for growth and resistance against these nanoparticles in the body.

MATERIALS AND METHODS

Animals

Seventy adult, male Wistar rats (weighing 230±20 g and 8 weeks old) were purchased from the animal house of Shiraz University of Medical Sciences and kept in the same place for 2 weeks before starting the study to adapt to the environment. During this study, the animals were kept in suitable conditions with a controlled temperature of 23±2 °C, 12 hours of light/darkness and 50-60% moisture. The animals were placed in special transparent macrolone cages with dimensions of 20×30×55 cm^3 with a retractable and movable steel roof. Five rats were kept in each cage. The animal food was in the form of compact food or pellets purchased from Daniran Livestock and Poultry Company in Shiraz. Access to adequate water and food was free during the study. Therefore, the storage conditions were considered same for all animals. The ethical and experimental protocol of this study of working with laboratory animals was approved by the Medical Ethics Review Committee of Shiraz University of Medical Sciences (Ethical code number: A1732-20) and it was observed until the last phase of the study.

The study protocol

In this experimental study, 70 adult, male Wistar rats were grouped in 7 groups of 10 completely randomly. The animals in the control group (group 1) did not receive any treatment during the study period. In the Ni50 group (group 2), animals received 50 mg/kg NiNPs (Pishgam Iranian Group, Iran) by oral gavage for 28 days. Animals in the Ni50+GA150 group (group 3) received 50 mg/kg NiNPs and 150 mg/kg GA (Merck, Germany) by oral gavage for 28 days. In the Ni50+GA300 group (group 4), the animals received 50 mg/kg NiNPs and 300 mg/kg GA by oral gavage for 28 days. Animals in the Ni50+Cur150 group (group 5) received 50 mg/kg NiNPs and 150 mg/kg Cur (Merck, Germany) by oral gavage for 28 days. In the Ni50+Cur300 group (group 6), animals received 50 mg/kg NiNPs and 300 mg/kg Cur by oral gavage for 28 days. Animals in the Ni50+GA300+Cur300 group (group 7) received 50 mg/kg of NiNPs, 300 mg/kg of GA, and 300 mg/kg of Cur by oral gavage for 28 days, respectively. Doses of NiNPs, GA and Cur were administered daily at 8 am, 12 pm and 4 pm, respectively, in all study groups. The selection of NiNPs, GA and Cur doses was determined and prescribed based on the previous studies (Marzban et al. 2020; Rong et al. 2018; Wang et al. 2015). At the last phase of the study, the samples of blood were taken from all animals and serum levels of hepatic transaminases (ALT and AST), blood plasma parameters (Glucose, TP, Bil, Alb, Cr, BUN, triglyceride, cholesterol, HDL, LDL and ALP) and pituitary-testicular axis hormones (FSH, LH, testosterone and dihydrotestosterone) were measured.

Blood parameters analysis

At the last phase of the study, all the animals were anesthetized with ether (Merck, Germany) after 11 hours of fasting, in one day (Wang et al. 2010). All animals were placed in anesthesia Jar with ether-impregnated cotton and were anesthetized. The reason for using ether was that ether anesthesia is mild and has little effect on blood flow velocity. On the other hand, it is less dangerous to breathe than chloroform. After anesthetizing the animals, blood samples were drawn from the left ventricle of the animals without opening the chest using 5 ml syringes. The samples of blood were placed in an incubator at 37 ° C to complete the agglutination process. Blood samples were then centrifuged at 3500 rpm (MSE, England) for 10 minutes to separate the serum. The obtained serum was stored in the freezer at -20 °C until the assessment of serum levels of hepatic transaminases, blood plasma parameters and pituitary-testicular axis hormones.

The glucose oxidase method was used to measure the plasma glucose levels (El-Borady et al. 2020) and

according to the instructions of the glucose measuring kit (Pars Azmoun, Iran). Serum level of total Bil was measured by photometric method using Diazo 2 and 4 Dichloroaniline (DCA) according to the manufacturer instructions (Pars Azmoun, Iran). Serum Cr level was measured using JAFFE method according to the instructions of Cr assay kit (Pezhuhan Teb, Iran). Serum BUN level was measured by DCA method according to the manufacturer's instructions (Pars Azmoun, Iran). Serum levels of triglyceride (Pars Azmon, Iran), cholesterol (Man Company, Iran), HDL (biochemistry, Iran) and LDL (Pars Azmon) were measured using the enzymatic method and according to the manufacturer's kits. Serum levels of FSH (BT Lab, China), LH (BT Lab, China), testosterone (IBL, Germany) and dihydrotestosterone (IBL, Germany) were measured using ELISA kits according to the manufacturer's instructions.

Serum levels of AST, ALT, ALP, Alb and TP were measured using the RA-1000 auto-analyzer (Technicon, USA) and the manufacturer's instructions were followed (Pars Azmoun Company, Iran). Serum ALT and AST levels were measured by IFCC (International Federation of Clinical Chemistry) method without adding Pyridoxal-50phosphate. Serum ALP level was measured using the PGKC method (Deutsche Gesellschaft Fur Klinische Chemie). Also, based on Biuret method, serum TP level was measured using Photometric method and serum Alb level was measured by BCG method (Bromocresol-Green) (Farashbandi et al. 2021).

Statistical analysis

The data resulting from the measurement of serum levels of liver transaminases, plasma blood parameters and pituitary-testicular hormones were analyzed by SPSS software version 20 (SPSS Inc, Chicago, IL, USA) and were analyzed using one-way ANOVA method followed by Tukey's *post-hoc* test to compare the mean data. The obtained values were reported as Mean±SEM (standard error of the mean) and a significant level of P <0.05 was considered. The Figures were drawn by GhraPhpad software version 5 (FigurePad Prism, Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

The results of this study indicated that glucose levels (Figure 1) in Ni50 group have increased significantly compared to the control group ($p<0.05$). NiNPs administration has been shown to be associated with high fasting blood sugar and insulin levels. The evidence suggests that exposure to nickel has a greater effect on glucose level and hyperglycemia induction than other divalent metals by increasing glycogenolysis and altering hepatic gluconeogenesis. Treating rats with nickel increases liver lipid peroxides and decreases the activity of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx) as well as hepatic glutathione (GSH) concentrations.

Nickel has been reported to reduce glucose regulation by increasing ROS, thereby impairing insulin function (Liu et al. 2015). In Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups, glucose levels showed a significant increase compared to the control group ($p<0.05$), in contrast, compared to Ni50 group, glucose level decreased significantly ($p<0.05$). Also, in Ni50+Cur300 and Ni50+GA300+Cur300 groups, a significant decrease in glucose levels was observed compared to Ni50 group ($p<0.05$), while in comparison with the control group, no significant difference was observed ($p>0.05$). Natural products and their derived compounds have a long history of clinical use as well as better patient tolerance and acceptance. Curcumin and GA have been shown to inhibit oxidative stress and inflammation (Dludla et al. 2018; Hashemzaei et al. 2020). In the present study, co-administration of GA and Cur at a dose of 300 mg/kg improved glucose levels compared with the control group. Synergistic effects between different compounds may lead to increased pharmacological effects (Pujimulyani et al. 2020).

Co-administration of GA and Cur in rats treated with NiNPs seems to enhance each other's effects compared to administrating them separately. The positive effects of Cur have been reported to increase the sensitivity of cells to insulin. Curcumin leads to increased insulin secretion due to prevention of apoptosis and oxidative stress along with increased activity of antioxidant enzymes. On the other hand, studies show that Cur is associated with increased glucose uptake and utilization by skeletal muscle cells and adipocytes, and the inhibition of gluconeogenesis (Panda et al. 2021). Gallic acid has been shown to improve insulin sensitivity since it regulates the expression of hepatic insulin signaling proteins such as insulin receptor, insulin receptor substrate 1 and phosphatidylinositol-3 kinase as demonstrated in mice fed with a high-fat diet. Gallic acid also reduces the expression of proteins associated with hepatic gluconeogenesis such as fructose 1,6-bisphosphatase and regulates the expression of hepatic glycogen synthase (Variya et al. 2020; Huang et al. 2016). The results of these studies are consistent with the results in improving glucose levels.

In this study, triglyceride level (Figure 2A) in Ni50 group was significantly higher than that of the control group ($p<0.05$). The levels in Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups were also higher than that in the control group ($p<0.05$), but lower

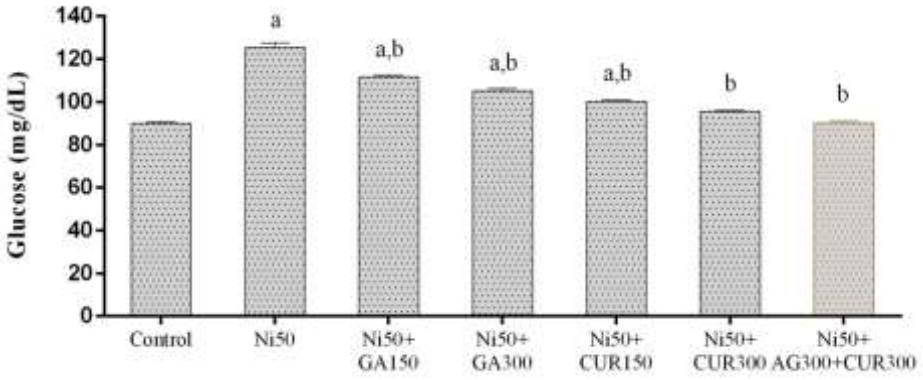

Figure 1. Comparison of mean and SEM of the serum glucose levels in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

than that in the Ni50 group ($p<0.05$). Also, in Ni50+Cur300 and Ni50+GA300+Cur300 groups, a significant lower level was observed as compared to Ni50 group ($p<0.05$), but not to the control group ($p>0.05$). The effects of the treatment on either cholesterol or LDL were similar to those on triglyceride, except that in Ni50+Cur300 group, the levels were significantly higher than that in control and Ni50+GA300+Cur300 groups ($p<0.05$) (Figure 2B and 2C). HDL level (Figure 2D) in the Ni50 and Ni50+GA150 groups showed a significant decrease compared to the control and Ni50+GA300+Cur300 groups ($p<0.05$). In Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 groups, HDL level showed a significant decrease compared to the control and Ni50+GA300+Cur300 groups ($p<0.05$). In contrast, HDL level showed a significant decrease compared to the Ni50 group ($p<0.05$).

Previous studies show that the administration of NiNPs in rats for 28 days increases triglyceride and LDL and decreases HDL compared to the control group, which is consistent with the results of this study (Ali et al. 2021). Increased triglyceride levels appear to be associated with increased levels of LDL, which is responsible for transporting cholesterol to the blood, while HLD, which is responsible for transporting cholesterol to the liver, is significantly reduced (Abdel-Ghafarf et al. 2018). It has been shown that, regular consumption of Cur for 12 weeks in patients with metabolic syndrome lowered triglyceride, LDL and total cholesterol levels, but not HDL levels (Yang et al. 2014). Also, Cur reduces serum lipid levels in rats by affecting fatty acid metabolism (Xia et al. 2020). Curcumin can inhibit expression of LDL receptor gene by activating Peroxisome proliferator- activated receptor gamma (PPAR-γ). In addition, it appears that Cur can affect the synthesis and catabolism of triglyceride-rich lipoproteins and cholesterol metabolism pathways. Thus, Cur appears to play an important role in reducing plasma triglyceride and cholesterol concentrations by reducing the expression of lipogenic genes (Jalali et al. 2020). It has been reported that the administration of Gallic acid at a dose of 100 mg/kg alone for 4 weeks in rats has no effect on triglyceride, HDL and cholesterol levels, however it does reduce LDL levels. Also, the co-administration of GA and Cur in this study had no effect on triglyceride, HDL and cholesterol levels but reduced LDL levels (Abarikwu et al. 2016). The lack of significant reductions in triglyceride, HDL and cholesterol levels contradicts the results of our study. Using various experimental models, it has been shown that the administration of GA for 4 weeks improves lipid profile, antioxidant status and insulin resistance in rats with a high-fat diet (Dludla et al. 2018).

The NiNps caused severe liver damage as shown by the profound increase of ALT, AST, ALP and Bil, and decrease of Alb and TP ($p<0.05$) (Figure 3A, 3B, 3C, 3D, 3E and 3F, respectively). The protective effect of Cur and GA was obvious as all the hepatoxic markers were significantly lower of higher in Ni50+GA150, Ni50+GA300 and Ni50+Cur150 groups than in the Ni50 group. Evaluating the hepatotoxicity of NiNPs in rats with different doses of nanoparticles increased liver functional enzymes (ALT, AST and ALP) and decreased SOD, GPx and Cat (Abdulqadir & Aziz 2019). In rats, NiNPs caused significant increases of Malondialdehyde levels in liver and kidney tissues and decrease of Glutathion level and SOD activity, indicating oxidative stress conditions. These changes were attributed to the overproduction of ROS caused by NiNPs, which leads to altered antioxidant activity at the level of gene and protein expression. The decreases in TP and Alb levels as observed in this study could be associated with the inhibition of protein expression. In addition, overproduction of ROS can damage cell membranes which in turn resulted in the increase of hepatic transaminases (Ali et al. 2021).

Curcumin is believed to be unique because it has both β-diketone and phenolic hydroxyl groups in one

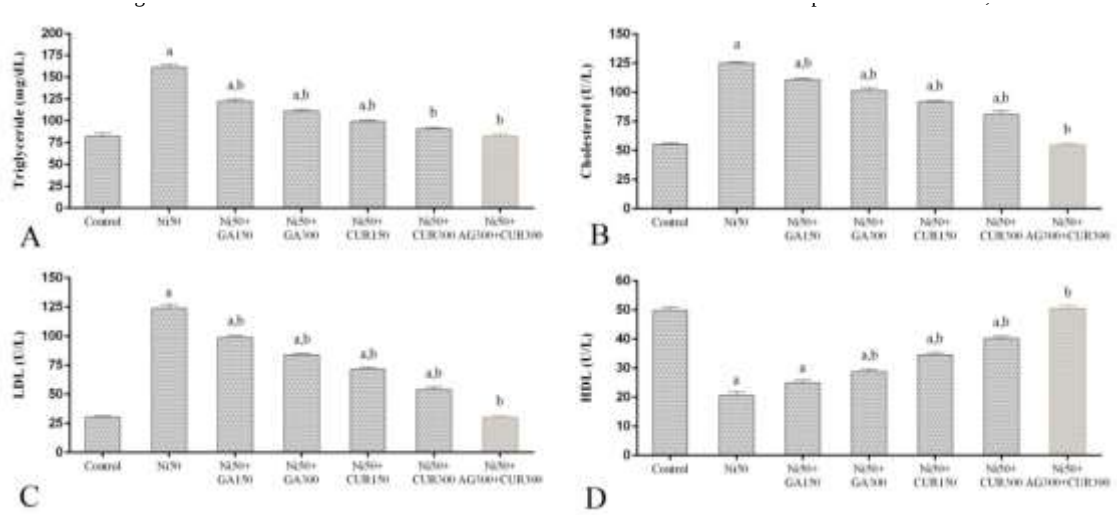

Figure 2. Comparison of mean and SEM of the serum triglyceride (A), cholesterol (B), LDL (C) and HDL (D) levels in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

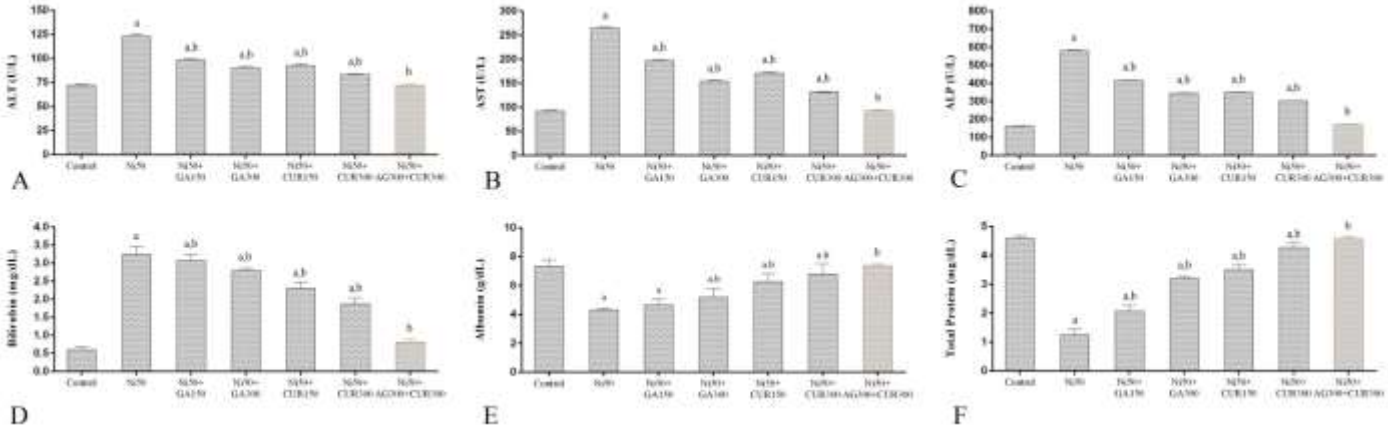

Figure 3. Comparison of mean and SEM of the serum levels of ALT (A), AST (B), ALP (C) and Bil (D), Alb (E) and TP (F) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

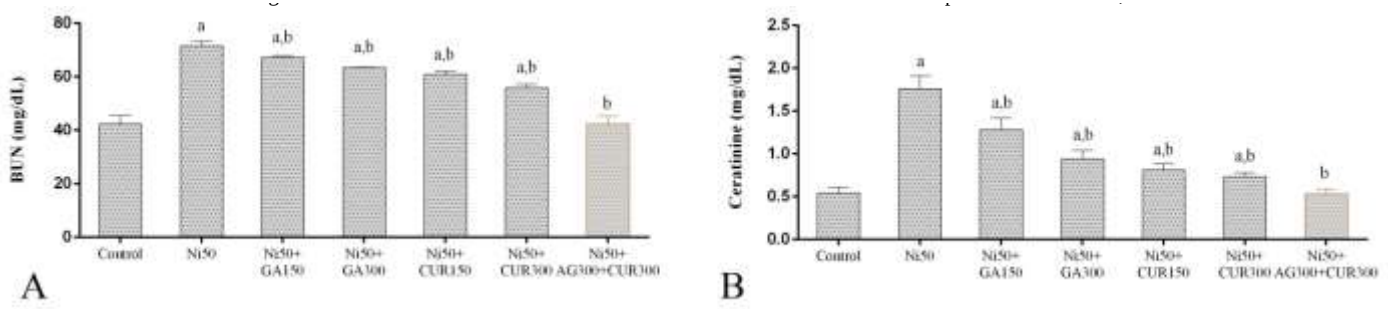

Figure 4. Comparison of mean and SEM of the serum levels of BUN (A) and Cr (B) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

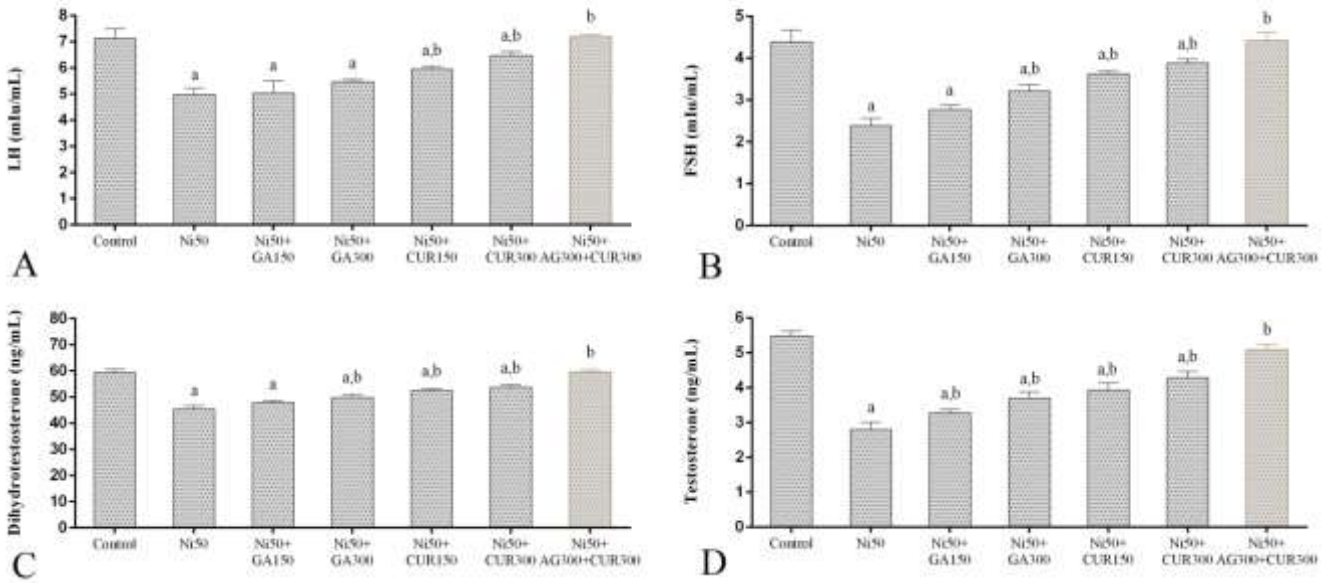

Figure 5. Comparison of mean and SEM of the serum levels of LH (A), FSH (B), testosterone (C) and dihydrotestosterone (D) in control, Ni50, Ni50+GA150, Ni50+GA300, Ni50+Cur150, Ni50+Cur300 and Ni50+GA300+Cur300 groups. a and b $p<0.05$, as compared with control and Ni50 groups, respectively. Ni: Nickel nanoparticles, GA: Gallic acid, Cur: Curcumin

molecule. This antioxidant molecule by trapping and stabilizing free radicals, especially lipid peroxyl radicals, can prevent the spread of oxidation and thus prevent tissue damage (Sökmen & Khan 2016). Studies showed that Cur has antioxidant and anti-inflammatory properties by suppressing NF-κβ and reducing oxidative stress and inflammation (Pulido-Moran et al. 2016). In addition, it can enhance the activity of antioxidant enzymes such as SOD and GPx by scavenging free radicals. This result suggests that Cur can be effective in protecting the liver by reducing oxidative stress (Jalali et al. 2020). Gallic acid has been reported to be effective in improving hepatic parameters induced by transient ischemia. Serum levels of ALT, AST, ALP and Bil showed a significant decrease with the administration of 50 and 100 mg/kg GA compared to the transient ischemia group (Akbari et al. 2019).

Previous studies on the effects of GA on liver have also shown that it protects liver tissue from damages caused by transient ischemia, Paracetamol and CCl4 by inhibiting ROS and exerting antioxidant activity (Wang et al. 2014; Bayramoglu et al. 2015) The research shows that GA can be an inhibitor of lipid peroxidation. The levels of malondialdehyde and lactate dehydrogenase have significantly increased in cells exposed to NiSO4, while treatment of these cells with Gallic acid has reduced the levels of malondialdehyde and lactate dehydrogenase. The studies have confirmed that GA, produces a free radical system consisting of hydroxyl radical and xanthine oxidase through the Fenton reaction, which leads to the removal of the free radical anion superoxide and it also has an inhibitory effect on the oxidation of cytochrome P450 3A (CYP3A) microsomal human liver to reduce tissue accumulation of ROS. In inducing apoptosis, GA, predominantly has a prooxidative effect and significantly protects cells against NiSO4-induced oxidative stress (An et al. 2016).

Blood urea nitrogen and creatinine are important kidney indicators. Their changes indicate the extent of kidney damage. In the present study, the administration of NINPs increased serum levels of BUN and Cr (Figure 4A and 4B, respectively) in Ni50 group compared to control group ($p<0.05$). Serum levels of BUN and Cr showed a significant increase in Ni50+GA150, Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 ($p <0.05$), in contrast, they showed a significant decrease compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, a significant increase in BUN and Cr levels was observed compared to Ni50 group ($p<0.05$), while in comparison with the control group, no significant difference was observed ($p>0.05$).

Nickel nanoparticles cause the accumulation of inflammatory cells and tissue damage in the kidney, and the inflammatory response in the kidney indicates damage to the filtration capacity of the kidney, and an increase in Cr level is the best confirmation for the kidney damage (Hendi 2011). Creatinine and urea are byproducts of cellular metabolism that are mainly excreted by the kidneys. Renal impairment increases plasma Cr and urea. Therefore, the increase in both products in the Nickel-treated group may be due to the nephrotoxic effects of Nickel on renal cells. Nickel is a nephrotoxin (Yin et al. 2021) and NiNPs increase serum Cr and BUN levels significantly, indicating impaired renal function (Ali et al. 2021). Other studies have reported significant changes in serum BUN and Cr levels after exposure to NiNPs (Ali 2019). This increase may be due to the accumulation of Nickel in the kidney tissue, which may impair the filtration of urea and Cr and increase them in the blood (Dumala et al. 2018).

The administration of Cur in ischemia-induced dysfunction and oxidative stress induced in kidney tissue of rats significantly reduced Cr and BUN. It has been shown that the use of Cur leads to a relative improvement in renal function as well as a reduction in oxidative stress and leukocyte infiltration due to ischemia. Some researchers have shown that Cur can directly inhibit chemokines. In addition, it has been suggested that Cur induces the expression of co-oxygenase-1 in renal epithelial cells, which is also a protective mechanism against oxidative stress. Curcumin has also been shown to inhibit TNF-α by stimulating co-oxygenase-1, thereby preventing leukocyte infiltration (Najafi et al. 2015). The results show that pretreatment with GA as an antioxidant reduces serum malondialdehyde levels and increases GSH levels and GPX activity in the kidney. In addition, GA pretreatment reduces urea and Cr levels (Ahmadvand et al. 2019). ROS or reactive nitrogen species (RNS) production is the major cause of kidney damage due to treatment with NiNPs. ROS is involved in the pathophysiology of NiNPs by inducing apoptosis, increasing lipid peroxidation, and activating cellular stress signaling pathways (Genchi et al. 2020). Antioxidants such as SOD, CAT, GPX and GSH are responsible for protecting cells against free radicals and oxidative stress. Gallic acid as a polyhydroxyphenolic compound is able to eliminate ROS. Oxidative stress caused by NiNPs leads to damage to macromolecules due to lipid peroxidation, DNA oxidation, protein oxidation, enzyme inactivation and dysfunction of various membranes. Glycolic acid reduces these destructive events by increasing the enzymatic activity of antioxidants such as GSH and GPX (Ahmadvand et al. 2019).

Serum levels of pituitary-testicular hormones play an important role in the process of spermatogenesis and fertility. Any change in the levels of these hormones can cause fertility problems. In this study, LH hormone levels (Figure 5A) in the groups of Ni50, Ni50+GA150 and Ni50+GA300 showed a significant decrease

compared to the control group ($p<0.05$). In Ni50+Cur150 and Ni50+Cur300 groups, serum LH levels showed a significant decrease compared to the control group ($p<0.05$), in contrast, compared with the Ni50 group showed a significant increase ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum LH level was observed in comparison with Ni50 group ($p<0.05$) while in comparison with the control group, no significant difference was observed ($p>0.05$). Serum levels of FSH and dihydrotestosterone (Figure 5B and 5C, respectively) in Ni50 and Ni50+GA150 groups showed a significant decrease compared to the control group ($p<0.05$). In Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 groups, serum levels of FSH and dihydrotestosterone showed a significant decrease ($p <0.05$) compared to the control group, but in contrast, showed a significant increase compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum levels of FSH and dihydrotestosterone was observed in comparison with the Ni50 group ($p<0.05$) while in the control group, no significant difference was observed ($p>0.05$). Serum testosterone level (Figure 5D) in Ni50 group showed a significant decrease compared to the control group ($p<0.05$). Serum testosterone levels in Ni50+GA150, Ni50+GA300, Ni50+Cur150 and Ni50+Cur300 showed a significant decrease compared to the control group ($p<0.05$), in contrast, they showed a significant increase compared to the Ni50 group ($p<0.05$). Also, in Ni50+GA300+Cur300 group, an increase in serum testosterone levels was observed compared to the Ni50 group ($p<0.05$), while no significant difference was observed compared to the control group ($p>0.05$). Decreased circulating LH, FSH, testosterone and dihydrotestosterone after treatment with NiNPs may be related to two mechanisms. Firstly, nanoparticles are able to affect the pituitary-testicular axis by altering sex hormone secretion and cellular activity (Forgacs et al. 2012). Secondly, NINPs may alters gene expression in the testis. In a study on the effect of NiNPs density on Zebrafish reproduction, it was found that the higher the density, the more severe the damage the caused on fertility (Ispas et al. 2009). These findings were in agreement with the present study. Nickel nanoparticles have been shown to produce oxidative stress that induces plasma membrane peroxidation, DNA fragmentation, mitochondrial membrane change and sperm morphology (Gallo et al. 2016).

The positive effects of Cur on improving the levels of pituitary-testicular hormones appear to be due to its protective effects on Leydig cells. Curcumin probably exerts its protective effects due to its anti-apoptotic, antioxidant and anti-genotoxic properties. In addition, Cur inhibits cortisol secretion by suppressing adrenocorticotropic hormone and increasing mRNAs encoding steroid-controlling proteins (Mohamadpour et al. 2017). Oxidative stress is known to be a potent mediator of apoptosis. In this process, mitochondria are recognized as an important factor. Mitochondrial dysfunction due to oxidative stress can lead to the release of cytochrome *c* and activation of caspase, followed by cell death (Ilbey et al. 2009). The studies have shown that in the male mammalian reproductive system, LH stimulates Leydig cells to produce testosterone, which is important for initiating and maintaining spermatogenesis through the Sertoli cellular androgen receptor. In addition, FSH plays a crucial role in normal spermatogenesis in pubertal rats (Oyagbemi et al. 2016). The LH, FSH and testosterone are known as hormonal biomarkers of androgenic hormone (Adedara & Farombi 2013). In this study, the increase in FSH and LH concentrations was associated with a significant increase in testosterone and dihydrotestosterone levels in the GA-treated groups alone and in combination with Cur, indicating the androgenic potential of GA and Cur. These observations may indicate that GA has an effective stimulatory effect on the pituitary-testicular axis of adult male rats.

CONCLUSION

Treatment of rats with NiNPs at a dose of 50 mg/kg for 28 days caused liver damage as indicated by the changes in serum concentration of ALT, AST, glucose, total protein, bilirubin, albumin, Cr, BUN, triglyceride, HDL, LDL and ALP, and pituitary-testicular axis hormones (FSH, LH, testosterone and dihydrotestosterone). Curcumin at a dose of 150 or 300, or GA at a dose of 300 mg/kg, co-administered with the NiNPs, was effectively inhibited the toxicity of the nickle. The protective effect of Cur and GA is even greater when the two substances are administered simultaneously.

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