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The Effects of Copper Oxide Nanoparticles and Hydroalcoholic Extracts of Berberis Vulgaris, Descurainia Sophia and Silybum Marianum on Catalase, Glutathione Peroxidase, and Malondialdehyde Concentration in Male Diabetic Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Antioxidants can reduce the occurrence of long-term damages, caused by free radicals. Considering the importance of enhanced oxidative stress in the occurrence of nanoparticle-induced damages and effects of plant extracts on reducing oxidative stress, in this study, we aimed to evaluate the effects of copper oxide nanoparticles and hydroalcoholic extracts of Berberis vulgaris, Descurainia sophia, and silymarin on catalase, glutathione peroxidase, and malondialdehyde concentrations in male diabetic rats.

METHODS: In this experimental study, 50 Wistar rats (250-350 g) were divided in ten groups (five rats per group): healthy controls, healthy rats receiving nanoparticles, healthy rats receiving Berberis vulgaris, Descurainia sophia, and silymarin extracts (independently), diabetic controls, diabetic rats receiving copper nanoparticles, and diabetic rats receiving the extracts independently. In diabetic groups, diabetes was induced in half of the rats, using alloxan at a dose of 120 mg/kg. In addition to copper oxide nanoparticles, the control and diabetic groups independently received 0.5 cc of Berberis vulgaris, Descurainia sophia, and Silybum marianum extracts via intraperitoneal injection for 30 days. Then, the animals were anesthetized with ketamine and the liver tissues were removed. The concentrations of catalase, glutathione peroxidase, and malondialdehyde were measured and compared.

FINDINGS: In diabetic groups treated with copper nanoparticles, a significant increase was reported in the concentration of malondialdehyde (from 4.7±0.447 to 5.05±0.405). Moreover, a significant decline was observed in the activity of catalase enzymes (from 36.8±1.48 to 36.2±1.4832) and glutathione peroxidase (from 75.4±3.9115 to 72.4±4.3362). Based on the findings, Silybum marianum was more effective than Berberis vulgaris and Descurainia sophia in diminishing the effects of cooper nanoparticles.

CONCLUSION: The present results showed that the studied herbal extracts could be used for moderating the effects of oxidative stress, induced by copper oxide nanoparticles.

KEY WORDS: *Copper oxide nanoparticles, Herbal extracts, Diabetic rats, Catalase, Malondialdehyde, Glutathione peroxidase*.

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Introduction

Nanotechnology is capable of producing new materials, devices, and systems by the control of matter on atomic and molecular scales and manipulation of the properties of nanoscale materials. Conversion of materials into nanomaterials leads to changes in their chemical and biological properties and catalytic activities (1). Descurainia sophia belongs to the Brassicaceae family. The seed of this plant contains palmitic, linoleic, oleic, and stearic acids and is mainly used as a laxative or a modulator of body temperature in combination with cold water. In traditional medicine, Descurainia sophia has been used as an appetizer, stomach tonic, antipyretic agent, and laxative; in addition, this plant has been applied for the treatment of dyspepsia (2).

Berberis vulgaris (commonly known as barberry) is a member of the Berberidaceae family, containing berberine alkaloids, oxycontins, and berbamines. Overall, the amount of alkaloids in barberry root bark is higher than other parts of this plant (3). The components of Silybum marianum, including silymarin as the major therapeutic compound, are effective in reducing blood cholesterol. The leaves of Silybum marianum contain bitter and strong constituents, which are used for the treatment of anorexia and digestive failure. Also, glutathione in Silybum marianum plays a major role in liver detoxification. In general, silymarin mainly constitutes a group of compounds, called flavonolignans. A new form of Silybum marianum, known as silymarin-phosphatidylcholine complex, has been identified. This complex is absorbed by the body better than normal Silybum marianum. In clinical trials, this agent alone has been shown to be more effective in the treatment of hepatic disorders, compared to silymarin (4-6). According to the literature, the antioxidant effects of barberry on hepatocytes bear a similarity to silymarin, which is known as a protector of liver cells (7).

Diabetes denotes a group of metabolic disorders, characterized by hyperglycemia (8). A previous study showed that barberry exerts positive effects on the liver function of diabetic rats and is likely to prevent complications, associated with diabetes. This plant also helps regulate glucose homeostasis by decreasing glucose production and reducing oxidative stress (9). In a study by Lee and et al, it was demonstrated that berberine in barberry could reduce lipogenesis and suppress lipid peroxidation (10). Various in vitro studies have assessed the effects of silymarin on the culture medium and different cell types, indicating the antioxidant and anti-carcinogenic effects of this herbal extract (11).

Antioxidants such as quercetin and silymarin boost biological membranes and increase cell survival by stabilizing the membrane gangliosides. On the other hand, carcinogenic agents such as arsenic can cause malignancies in skin cells and induce oxidative stress; it has been shown that silymarin can fight these phenomena to some extent (12).

In this regard, Soto and et al, studied the effects of silymarin on the performance of pancreas in diabetic animals and showed the hypoglycemic and protective effects of flavonolignan in pancreatic tissues against damages (13). In addition, flavonoids such as silymarin, extracted from Silybum marianum, can reduce the glucose level and help regain normal weight through modulating liver enzymes, which are responsible for carbohydrate metabolism (via reducing the enzyme activity of liver phosphorylase and increasing the activity of glucokinase and glycogen synthase) (14). The aim of this study was to investigate the effects of copper oxide nanoparticles and Berberis vulgaris, silymarin, and Descurainia sophia extracts on catalase, glutathione peroxidase, and malondialdehyde (MDA) concentrations in male diabetic rats.

Methods

Materials: The materials used for preparing nanoparticles included Tris buffer, thiobarbituric acid, trichloroacetic acid, tetraethoxypropane, ethanol, Triton X-100, H_2O_2, sodium phosphate buffer, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), copper nitrate, citric acid, and ethylene glycol. Other materials used in this study were saline, ketamine, animal feed, test kits, and soluble silymarin (Gol Daru Co., Iran). The devices used in this experiment were as follows: TU-1901 double-beam UV-Vis spectrophotometer for X-ray diffraction (XRD), a UV-VIS spectrophotometer (6705 Series, Jenway Co.), a transmission electron microscope (TEM; JEM-200 CX), a scanning electron microscope (SEM; Model EM 902A, Zeiss), a centrifuge (Eppendorf Model 5810), a homogenizer (VMH-700), and an ultrasonic device (Parsonic 7500s, Pars Nahand, Iran). Experiments using SEM and TEM were performed in the laboratory of Razi Metallurgical Research Center of Karaj and XRD technique was utilized at the Department of Chemistry, Shiraz University of Medical Sciences, Iran.

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Preparation of hydroalcohlic extracts: The seeds of barberry and Descurainia sophia were collected in the flowering stage from Taft, Iran in September 2014. The herbarium numbers of barberry and Descurainia sophia (No. 949 and 755, respectively) were designated after species identification at the Faculty of Natural Resources, Yazd University of Medical Sciences, Iran. To prepare the hydroalcoholic extracts of barberry and Descurainia sophia, 200 g of plant seeds was separately washed and dried within 24 h in a dark room. The seeds were powdered, using an electric grinder. Then, 32 g of the powdered barberry and Descurainia sophia seeds was separately extracted with ethanol 100%, using a Soxhlet apparatus for 48 h at 50°C. Afterwards, the solvent was dried in a rotary device to produce the solid powder. The required volume of the powder was prepared, using double distilled water. The rotary device was employed for drying the solvent used for extraction. By increasing the evaporation time, solid powder could be prepared after complete drying of the solvent. Barberry and Descurainia sophia extracts (20 mg/kg), as well as silymarin (200 mg/kg, Gol Daru Co., Iran), were intraperitoneally injected (0.5 ml) in rats on a daily basis.

Synthesis of copper oxide nanoparticles: In order to synthesize copper oxide nanoparticles by sol-gel method, deionized water and ethanol (C_2H_5OH, > 99.9%, Merck, Germany) with a molar ratio of 1:1 (solvent), copper nitrate [$Cu(NO_3)_2.3H_2O$] (precursor solution), citric acid (complexing agent), and ethylene glycol (polymerization agent) were used, respectively. The prepared solution was stirred by a magnetic mixer at room temperature for one hour. An indirect bath heater was used to promote uniform heating. After reflux for 4 h at a temperature range of 90-110°C, a homogeneous solution was obtained. The dried gel was prepared after direct heating at a temperature of 120°C for 7 h and vaporizing the excess solvent from the green gel under infrared light. By placing the gel inside an oven for 1 hour at 160°C, the final powder containing nanoparticles was produced after grinding. The shape and entity of nanoparticles were studied through electron microscopic examinations and XRD technique.

Preparation of nanoparticle suspensions: To prepare the stock solution of nanoparticles, 10 g of nanoparticles was suspended in one liter of sterile medium. In order to disperse nanoparticles, an ultrasonic device (Parsonic 7500s, Pars Nahand, Iran) was employed for 30 min. In order to avoid errors, nanoparticle suspensions were prepared; the final concentration of the solution was 400 ppm. In this experiment, 0.5 ml of nanoparticles was intraperitoneally injected in each rat.

Experimental animals: In this study, 50 male Wistar rats, weighing 250-350 g (aged 8 weeks), were divided into ten groups of five rats: healthy controls, healthy rats receiving nanoparticles, healthy rats receiving barberry, Descurainia Sophia, and silymarin extracts (separately), diabetic controls, diabetic rats receiving copper nanoparticles, and diabetic rats receiving the extracts (separately). All the groups were intraperitoneally injected 0.5 cc of the extracts for 30 days. The rats were kept in polypropylene cages at a temperature of 22±1˚C and humidity of 60±10%, with 12 hours of light and 12 hours of darkness.

Diabetes mellitus induction (type 1 diabetes): Insulin-dependent diabetes mellitus was induced in rats with a single intraperitoneal injection of alloxan at a dose of 120 mg/kg of body weight; physiological serum was used as the solvent for alloxan (15). The criterion for diabetes was defined as increased blood glucose level (200-300 mg/dL) after one week of injection (16). All animal experiments were performed in accordance with the guidelines of the ethics committee. The rats in each group were identified by special marks. The rats only received water for 12 h, and alloxan was injected in the morning after 12 hours of fasting.

Measurement of catalase, glutathione peroxidase, and MDA: After diabetes induction and treatment for thirty days, the animals were anesthetized with ketamine and then sacrificed. The liver tissue was removed, rinsed with saline solution, dried, and then weighed. The tissues were homogenized (10%) with Tris buffer for 2 min, using a homogenizer and centrifuged at 3000 rpm. To prevent the destruction of enzymes and proteins, the entire process was carried out at 4°C. After centrifugation, the transparent solution was separated from the rest of the solution and used to measure the concentrations of MDA, glutathione peroxidase, and catalase enzymes. Measurement of MDA level was performed, using a method based on thiobarbituric acid reactive substances (TBARS) (17). Catalase activity was measured by a method proposed by Aebi and et al (18). In addition, glutathione peroxidase activity was evaluated, using a technique introduced by Rotruck and et al(19).

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Data extraction and analysis: The results are presented as Mean±SD. To examine the biochemical findings and compare the mean values in experimental groups, multivariate analysis of variance and Least Significant Difference (LSD) test were used. P-value less than 0.05 was considered statistically significant.

Results

Structural study of copper oxide nanoparticles:

Compared to light microscopes, objects appear much larger under TEM. The electron microscopic images are black and white, since some beams do not pass through the object and create black spots (fig 1). The layering of copper nanoparticles was confirmed, using SEM (fig 2).

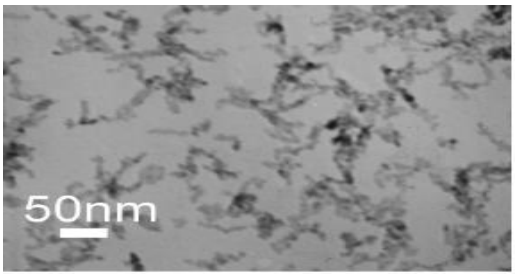


Figure 1. Transmission electron microscope (TEM) image of copper oxide nanoparticles

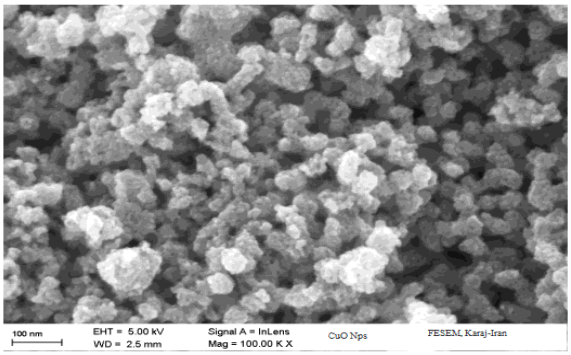


Figure 2. Scanning electron microscope (SEM) image of the layers of copper oxide nanoparticles

The diameter of copper oxide nanoparticles was estimated at 50 nm, using Scherrer equation and TEM. XRD is a widely used technique for analyzing crystal characteristics; in fact, XRD is utilized to study the characteristics of the samples. Also, XRD is used to determine crystal structure (i.e., network constant, network geometry, qualitative identification of unknown materials, determination of crystal phase, measurement of crystal size, crystal orientation, stress, tension, and network micro-deformations) (fig 3, 4).

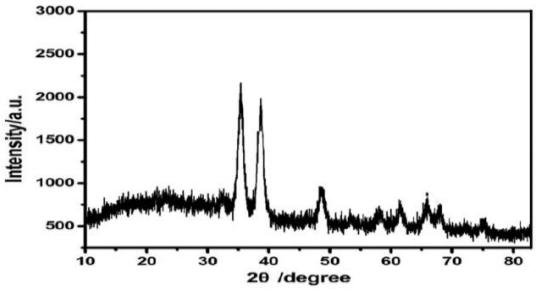


Figure 3. Image of X-ray diffraction (XRD) of copper oxide nanoparticles

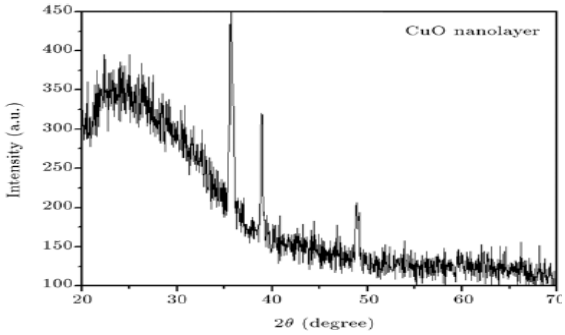


Figure 4. X-ray diffraction (XRD) graph of the layers of copper oxide nanoparticles

Catalase enzymes in different groups: The mean concentrations of catalase enzymes in groups receiving nanoparticles, barberry, Descurainia Sophia, and silymarin extracts were significantly different from the healthy control group ($p<0.05$). The mean concentration of this enzyme significantly decreased in the nanoparticle groups (healthy and diabetic), compared to the healthy control group, while the mean level significantly increased in groups receiving barberry, Descurainia Sophia, and silymarin. Groups receiving silymarin experienced the most significant increase in the concentration of catalase enzymes, compared to rats receiving barberry or Descurainia Sophia. Also, the mean concentration of catalase enzymes in diabetic rats receiving silymarin increased in comparison with the diabetic control group (fig 5).

Glutathione peroxidase in different groups: The mean concentration of glutathione peroxidase in groups receiving silymarin and nanoparticles was significantly different from the healthy control group ($p<0.05$). The mean concentration of glutathione peroxidase significantly reduced in the groups receiving nanoparticles (healthy and diabetic), while a considerable rise was reported in the silymarin group. Also, the mean concentration of glutathione peroxidase increased in diabetic rats receiving silymarin, compared to the diabetic control group (fig 6).

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MDA concentration in different groups: The concentration of MDA in the groups receiving nanoparticles and silymarin was significantly different from the healthy control group ($p<0.05$). MDA level significantly increased in the groups receiving nanoparticles (healthy and diabetic), while a significant decline was reported in groups receiving silymarin and barberry; the greatest reduction was reported in the silymarin group.

The mean MDA concentration in diabetic rats receiving silymarin and barberry significantly reduced, compared to the diabetic control group; the most significant decline was reported in rats receiving silymarin (fig 7).

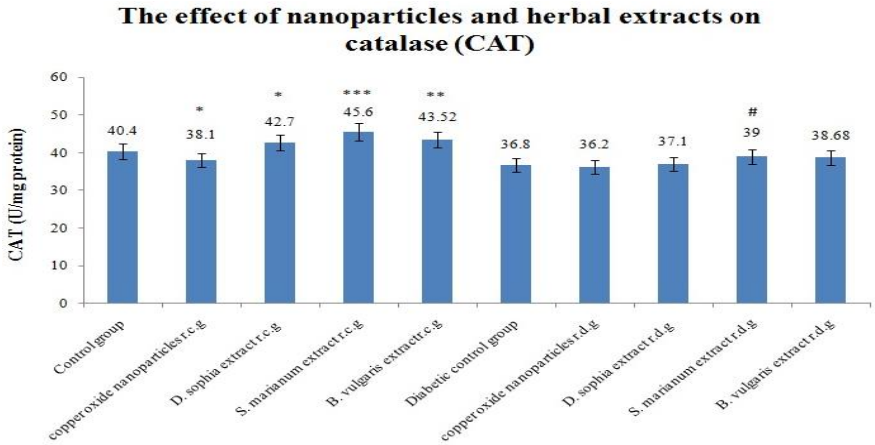


Figure 5. Concentration of catalase enzymes in the liver tissue of rats receiving herbal extracts and copper oxide nanoparticles

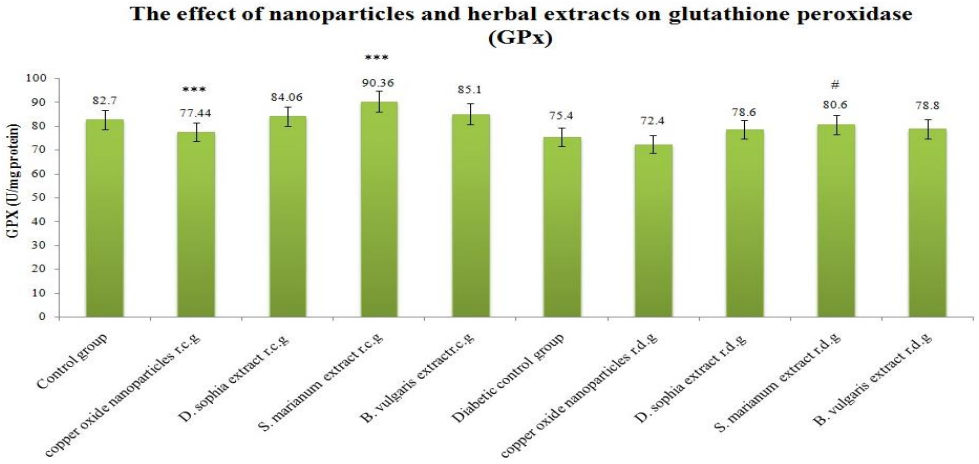


Figure 6. Glutathione peroxidase level in the liver of rats treated with herbal extracts and copper oxide nanoparticles

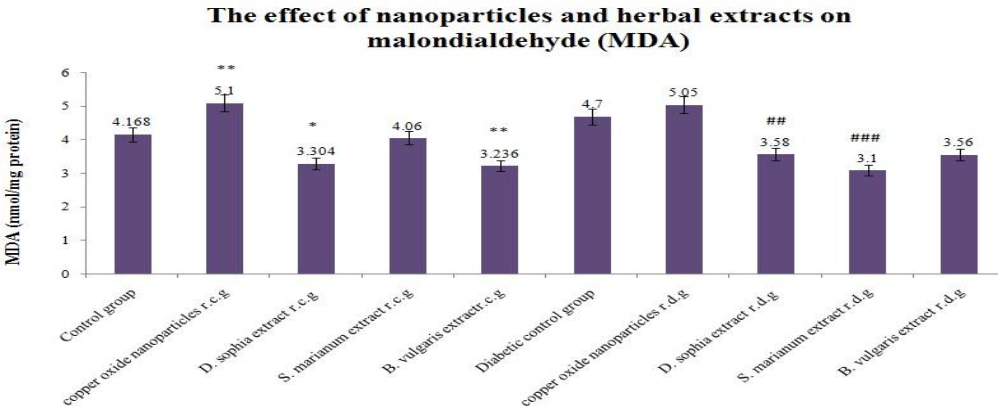


Figure 7. Malondialdehyde (MDA) concentration in the liver tissues of rats receiving herbal extracts and copper oxide nanoparticles. *Compared to the healthy control group, #Compared to the diabetic control group

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Discussion

In this study, copper oxide nanoparticles increased oxidative stress by raising the activity level of MDA enzymes. The extracts of silymarin, Berberis vulgaris, and Descurainia Sophia reduced the negative impacts of nanoparticles by raising the concentration of enzymes such as catalase and glutathione peroxidase; similar findings were reported in both healthy and diabetic rats. In a previous study, the activity level of antioxidant enzymes such as catalase and superoxide dismutase was higher in the liver of rats treated with barberry, compared to the control group. In consistence with the current research, this finding suggested the inhibitory effects of barberry on lipid peroxidation through increasing the level of antioxidant enzymes (20).

Moreover, in another study, use of copper oxide nanoparticles at a dose of < 50 nm reduced the levels of superoxide dismutase and catalase enzymes; this effect was intensified after one day and a week of intra-pulmonary injection (21). Liu and et al, showed that copper oxide nanoparticles could reduce the secretion of superoxide dismutase and catalase enzymes (22); the results were in alignment with the present study. Also, based on previous research, oxidative stress increases with respect to the toxicity of nanoparticles, and increased production of reactive oxygen species and oxidative stress can be considered as the markers of nanoparticle toxicity (21, 22). Various studies have assessed the effects of silymarin on the culture media and different cell types, implicating the antioxidant and anti-carcinogenic properties of this herbal extract (23); in fact, silymarin causes the differentiation of cells in the culture medium. On the other hand, silymarin has been shown to prevent uncontrolled cellular growth, cellular differentiation, and carcinoma by inhibiting tyrosine kinase receptors, which are activated by growth factors (24). Some flavonoids such as silymarin can increase antioxidant activity in the body and enhance the activity of antioxidant enzymes, which may be followed by decreased lipid peroxidation (25). The current results showed that silymarin, Berberis vulgaris, and Descurainia Sophia extracts could diminish the effects of oxidative stress, caused by nanoparticles in diabetic rats. Therefore, it is recommended to use these plant extracts, particularly silymarin, to moderate the adverse oxidative effects of nanoparticles, particularly in type 1 diabetic patients.

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