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The effects of ethanolic herbal extracts and CuO nanoparticles on catalase, glutathione peroxidase and malondialdehyde in male diabetic rats

Mohammad Mohammadifard[1] & Javad Mottaghipisheh[2]*

[1]*Department of Biology, Payame Noor University of Taft, Yazd, Iran; e-mail: mohamadm47@gmail.com*
[2]*Department of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran; e-mail: Imanmottaghipisheh@yahoo.com*

Abstract: Antioxidants can reduce the occurrence of long-term damages, caused by free radicals. In this study, we aimed to evaluate the effects of CuO nanoparticles and hydroalcoholic extracts of *Berberis vulgaris*, *Descurainia sophia* and *Silybum marianum* on catalase, glutathione peroxidase, and malondialdehyde concentrations in male diabetic rats. In case 50 Wistar rats (250–350 g) were divided in ten groups (five rats per group): healthy controls, healthy rats receiving nanoparticles, healthy rats receiving *B. vulgaris, D. sophia,* and *S. marianum* extracts (independently), diabetic controls, diabetic rats receiving CuO 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^{-1}. In addition to CuO nanoparticles, the control and diabetic groups independently received 0.5 ml of *B. vulgaris, D. sophia,* and *S. 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. In diabetic groups treated with CuO nanoparticles, a significant increase was reported in the concentration of malondialdehyde (from 4.7 ± 0.44 to 5.05 ± 0.40) (mean ± SD). Moreover, a significant decline was observed in the activity of catalase (from 36.8 ± 1.48 to 36.2 ± 1.48) and glutathione peroxidase enzymes (from 75.4 ± 3.91 to 72.4 ± 4.33). The results show that *S. marianum* extract was more effective than *B. vulgaris* and *D. sophia* extracts in diminishing the effects of CuO nanoparticles.

Key words: CuO nanoparticles; herbal extracts; diabetic rats; catalase; malondialdehyde; glutathione peroxidase

Abbreviations: CAT, catalase; MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); XRD, X-ray diffraction; TEM, transmission electron microscope; SEM, scanning electron microscope.

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 (Pajoumand & Shariat Tarbaghani 1998)

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, *D. 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 (Movahedian et al. 2011)

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 (Akkaya & Yılmaz 2012).

In a study by Lee et al. (2006), it was demonstrated that berberine in *B. vulgaris* could reduce lipogenesis and suppress lipid peroxidation.

The components of *Silybum marianum* (Asteraceae family) including silymarin as the major therapeutic compound are effective in reducing blood cholesterol. The leaves of *S. marianum* contain bitter and strong constituents, which are used for the treatment of anorexia and digestive failure. In general, silymarin mainly constitutes a group of compounds called flavonolignans.

A new form of silymarin, known as silymarin-phosphatidylcholine complex, has been identified. This

* Corresponding author

complex is absorbed by the body better than normal silymarin. In clinical trials this agent alone has been shown to be more effective in the treatment of hepatic disorders, compared to silymarin (Berkson 1999; Di Pierro et al. 2013; Sherif & Al-Gayyar 2013). According to the literature the antioxidant effects of *B. vulgaris* extract on hepatocytes bear a similarity to *S. marianum* extract, which is known as a protector of liver cells (Tsai & Tsai 2005). Diabetes denotes a group of metabolic disorders, characterized by hyperglycemia (Tripathi & Srivastava 2006). Various *in vitro* studies have assessed the effects of *S. marianum* extract on the culture medium and different cell types, indicating the antioxidant and anti-carcinogenic effects of this herbal extract (Soria et al. 2007).

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 (Kittur et al. 2002). With this regard Soto et al. (1998) studied the effects of *S. marianum* extract on the performance of pancreas in diabetic animals and showed the hypoglycemic and protective effects of flavonolignan in pancreatic tissues against damages. In addition flavonoids such as silymarin, extracted from *S. 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) (Toovey et al. 1981). On the other hand, the extract has been shown to prevent uncontrolled cellular growth, cellular differentiation and carcinoma by inhibiting tyrosine kinase receptors, which are activated by growth factors (Xiong et al. 2003). 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 (Kang et al. 2004).

In another study the use of CuO nanoparticles at a dose of < 50 nm reduced the levels of SOD and CAT enzymes; this effect was intensified after one day and a week of intra-pulmonary injection (Sandhya Rani et al. 2013). The aim of this study was to evaluate the intensifier effects of CuO nanoparticles and reductive effects of *B. vulgaris*, *S. marianum* and *D. sophia* extracts on oxidative stress by measuring of concentrations of catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) in male diabetic rats.

Material and 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 and ketamine. All the chemicals have been purchased from Merck Co. (Germany) and the animal feed has been prepared from Behparvar Co. (Iran). The devices used in this experiment were as follows: XRD (TU-1901 double-beam), UV-VIS spectrophotometer (6705 Series, Jenway Co.), transmission electron microscope (TEM; JEM-200 CX), scanning electron microscope (SEM; Model EM 902A, Zeiss), centrifuge (Eppendorf Model 5810), homogenizer (VMH-700) and an ultrasonic device (Parsonic 7500s, Pars Nahand, Iran).

Preparation of hydroalcoholic extracts

B. vulgaris, *D. sophia* and *S. marianum* seeds were collected in the flowering stage from Taft, Iran in September 2014. The herbarium numbers of *B. vulgaris*, *D. sophia* and *S. marianum* (No. 949, 755 and 647, respectively) were designated after species identification by Dr. Mohammad Dahestani at the Faculty of Natural Resources, Yazd University of Medical Sciences, Iran.

To prepare the hydroalcoholic extracts of these plants 200 g of seeds were separately washed and dried within 24 h in a dark room. The plants parts were powdered using an electric grinder. Then, 32 g of the powdered samples were 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 herbal extracts (20 mg kg^{-1}) were intraperitoneally injected (0.5 ml/day) for 30 days in the rats.

Synthesis of CuO nanoparticles

In order to synthesize CuO 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 [$Cu(NO_3)_2 \cdot 3H_2O$] (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 at a temperature range of 90–110 °C for 4 h, 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 at 160 °C for 1 h, 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 were suspended in one liter of sterile medium. For dispersing the nanoparticles an ultrasonic device was employed (Parsonic 7500s, Pars Nahand, Iran) 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 were 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 CuO nanoparticles, healthy rats receiving *B. vulgaris*, *D. sophia* and *S. marianum* extracts (separately), diabetic controls, diabetic rats receiving CuO 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 ℃ and humidity of 60 ± 10%, with 12 h of light and 12 h 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^{-1} of body weight; physiological serum was used as the solvent for alloxan (Gupta et al. 2005). The criterion for diabetes was defined as increased blood glucose level (200–300 mg/dl) after one week of injection (El Demerdash et al. 2005).

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 h of fasting.

Measurement of MDA, CAT and GPx
The animals were anesthetized with ketamine and then sacrificed after diabetes induction and treatment for 30 days. 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 ℃.

After centrifugation the transparent solution was separated from the rest of the solution and used to measure the concentrations of MDA, CAT and GPx enzymes. Measurement of MDA level was performed, using a method based on thiobarbituric acid reactive substances (TBARS) (Bagheri et al. 2011). CAT activity was measured by a method proposed by Aebi (1984). In addition, GPx activity was evaluated using a technique introduced by Rotruck et al. (1973).

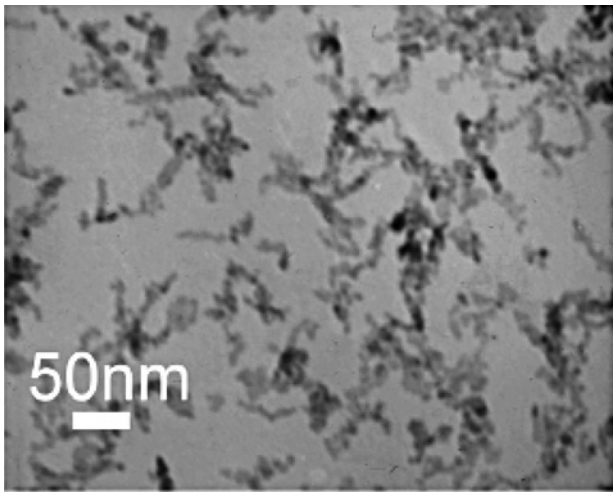

Fig. 1. Transmission electron microscope (TEM) image of copper oxide nanoparticles.

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 CuO nanoparticles
The diameter of CuO nanoparticles was estimated at 50 nm by using Scherrer equation and TEM (Fig. 1). Also, by using SEM the layering of CuO nanoparticles

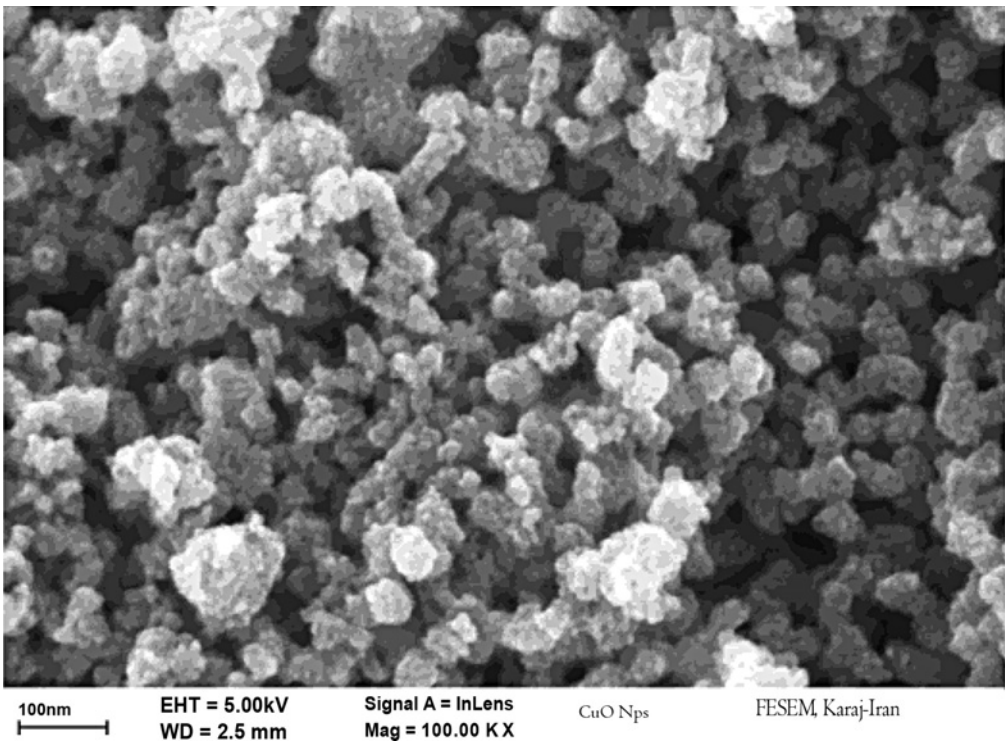

Fig. 2. Scanning electron microscope (SEM) image of the layers of copper oxide nanoparticles.

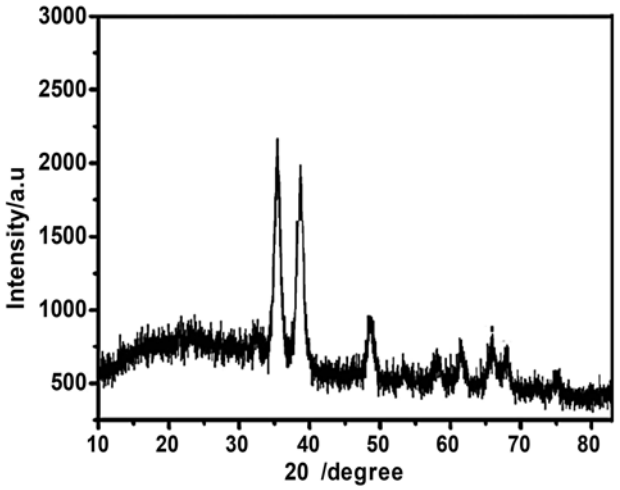

Fig. 3. Image of X-ray diffraction (XRD) of copper oxide nanoparticles.

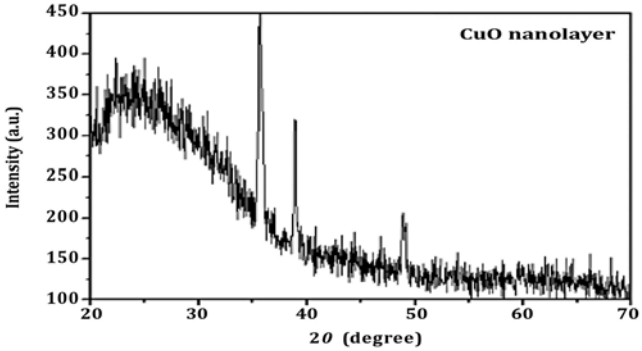

Fig. 4. X-ray diffraction (XRD) graph of the layers of copper oxide nanoparticles.

was confirmed (Fig. 2). XRD widely used to analyze of crystal characteristics. 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) (Figs 3, 4).

CAT concentration in different groups

The mean concentrations of CAT enzyme in groups receiving nanoparticles, *B. vulgaris*, *D. sophia*, and *S. marianum* 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 *B. vulgaris*, *D. sophia* and *S. marianum* extracts. Groups receiving *S. marianum* extract experienced the most significant increase in the concentration of CAT enzyme, compared to rats receiving *B. vulgaris* or *D. sophia* extracts. Also, the mean concentration of CAT enzyme in diabetic rats receiving *S. marianum* extract increased in comparison with the diabetic control group (Fig. 5).

GPx concentration in different groups

The mean concentration of GPx in groups receiving *S. marianum* extract and nanoparticles was significantly different from the healthy control group ($P < 0.05$). The mean concentration of GPx significantly reduced in the groups receiving nanoparticles (healthy and diabetic), while a considerable rise was reported in the *S. marianum* extract group. Also, the mean concentration of GPx increased in diabetic rats receiving *S. marianum* extract, compared to the diabetic control group (Fig. 6).

MDA concentration in different groups

The concentration of MDA in the groups receiv-

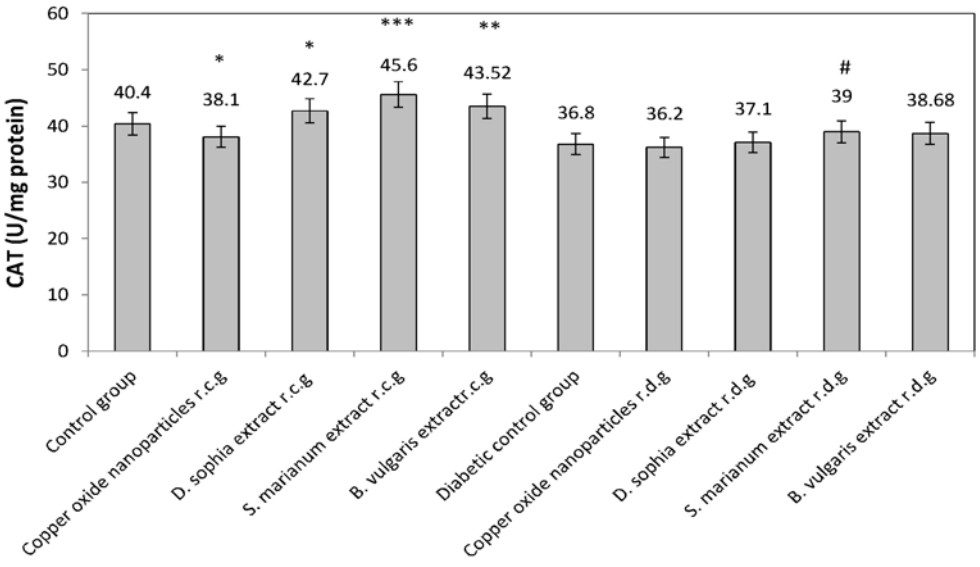

Fig. 5. Concentration (mean ± SD) of CAT enzyme in the liver tissue of rats receiving herbal extracts and CuO nanoparticles. *Compared to the healthy control group; #compared to the diabetic control group. r.c.g: receiver control group; r.d.g: receiver diabetic group.

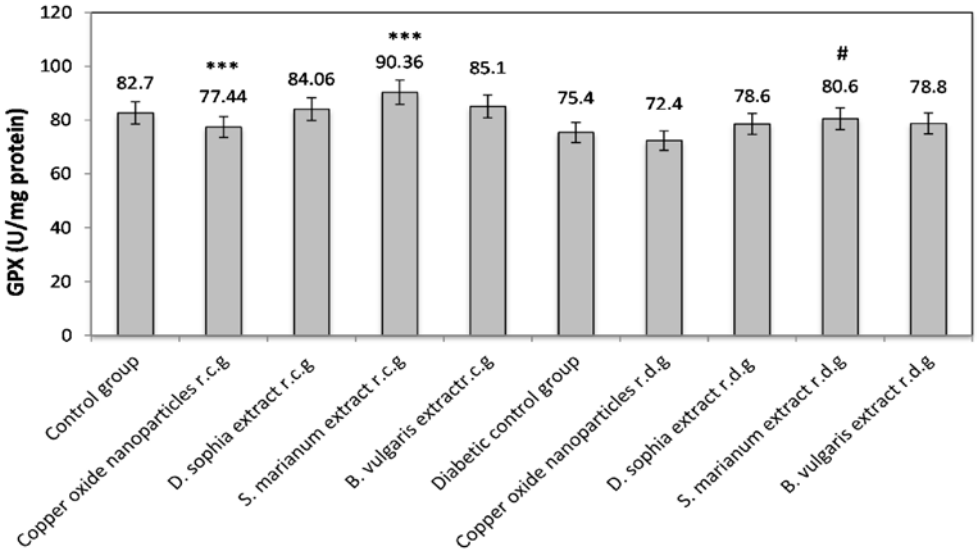

Fig. 6. Concentration (mean ± SD) of GPx enzyme in the liver tissue of rats receiving herbal extracts and CuO nanoparticles. *Compared to the healthy control group; #compared to the diabetic control group. r.c.g: receiver control group; r.d.g: receiver diabetic group.

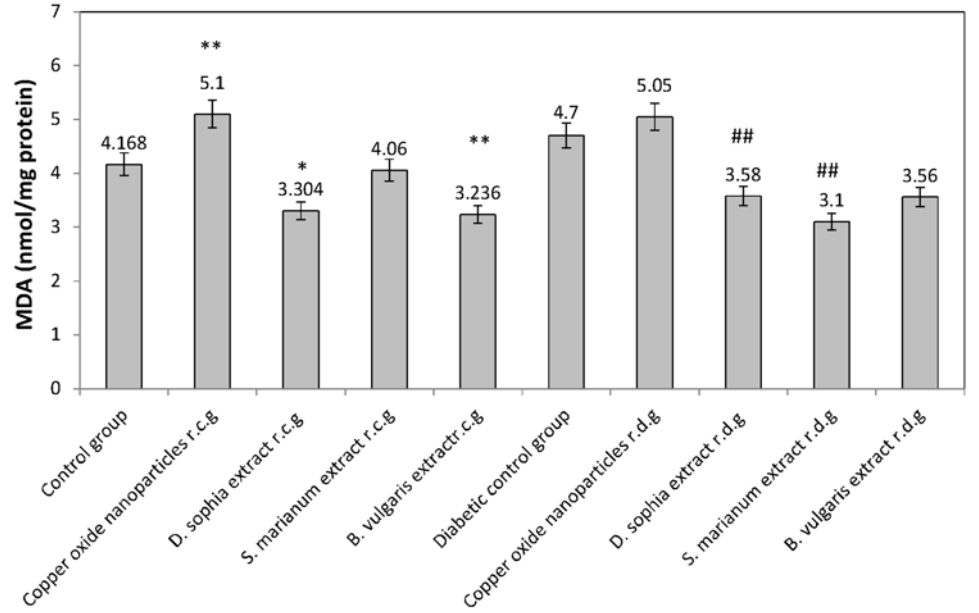

Fig. 7. Concentration (mean ± SD) of MDA enzyme in the liver tissue of rats receiving herbal extracts and CuO nanoparticles. *Compared to the healthy control group; #compared to the diabetic control group. r.c.g: receiver control group; r.d.g: receiver diabetic group.

ing nanoparticles and *S. marianum* extract 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 *S. marianum* and *B. vulgaris* extract; the greatest reduction was reported in the *S. marianum* group. The mean MDA concentration in diabetic rats receiving *S. marianum* and *B. vulgaris* extract significantly reduced, compared to the diabetic control group; the most significant decline was reported in rats receiving *S. marianum* extract (Fig. 7).

Discussion

In a previous study the activity level of antioxidant enzymes such as CAT and superoxide dismutase (SOD) was higher in the liver of rats treated with *B. vulgaris* extract, compared to the control group. In consistence with the current research, this finding suggested the inhibitory effects of *B. vulgaris* extract on lipid peroxidation through increasing the level of antioxidant enzymes (Murugesh et al. 2005).

In addition, it was shown that *B. vulgaris* extract 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 (Singh & Kakkar 2009).

Liu et al. (2011) demonstrated that CuO nanoparticles could reduce the secretion of SOD and CAT enzymes; 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 (ROS) and oxidative stress can be considered as the markers of nanoparticle toxicity (Sandhya Rani et al. 2013; Liu et al. 2011).

In the present research, we have investigated the harmful effects of CuO nanoparticles by increasing oxidative stress and reducing effects of *S. marianum*, *B. vulgaris* and *D. sophia* extracts on it in both of control and diabetic groups of rats. For this purpose, we evaluated the concentrations of CAT, GPx and MDA. CuO nanoparticles increased oxidative stress by raising the activity level of MDA enzyme. The extracts of *S. marianum*, *B. vulgaris* and *D. sophia* reduced the negative impacts of nanoparticles.

By evaluating and comparing of the levels of the enzymes we can conclude about the effects of CuO nanoparticles and the extracts. For example, the concentration of MDA in control and nanoparticle is 4.1 and 5.1 nmol mg^{-1} protein, respectively. While the MDA concentrations in receiver groups of the herbal extracts are less than of 4.1 nmol mg^{-1} protein. So we can compare and result about this effects. By raising the concentration of enzymes such as CAT and GPx, similar findings were reported in both healthy and diabetic rats.

The current results showed that *S. marianum*, *B. vulgaris* and *D. 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 *S. marianum* extract to moderate the adverse oxidative effects of nanoparticles, particularly in type 1 diabetic patients.

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