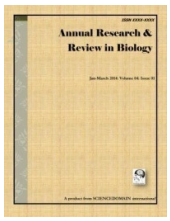

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Effects of Silymarin on Oxidative Stress Markers in Rats Treated with Magnesium Oxide Nanoparticles

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the protective and anti-oxidative effects of silymarin on rats treated with MgO NPs. Number of 32 male wistar rats were used in this experiment, assigned to four groups; one, the control group and the three others were treatment groups. The treated groups inclusive of a group receiving 400 ppm Mg NPs, a group received 200 mg/kg silymarin, and a group received silymarin and that dose of Mg NPs. All groups received the treatment peritoneally as long as four weeks uninterruptedly. Post-treatment tissue level of malondialdehyde as well as the activities of catalase, glutathione peroxidase and superoxide dismutase were measured in the liver. The data were analyzed by implementation of one-way ANOVA and Bonferroni tests. In animals treated with MgO NPs, MDA level significantly increased (P=0.000), while a significant decrease was observed in GPX (P=0.000), SOD (P=0.001), and CAT (P=0.008) activities. However, silymarin prevented the effect of them in the group receiving silymarin and MgO NPs significantly and caused an increase in GPX, SOD and CAT activities as well as a decrease in MDA level (P<0.05). As, nanoparticles have harmful effects on the body raised from the generation of reactive oxygen species, this study showed that silymarin, with its antioxidant properties, may decrease free radicals produced by MgO NPs.

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1. INTRODUCTION

Despite of the fact that nanotechnology may improve the current knowledge on the Nanoscale [1], these materials leave harmful effects on biological environment. Nanoparticles can be directly transmitted into the body via water, food, cosmetics and medicines. Metal oxide nanoparticles have a large scale industrial, medical and domestic applications [2]. The growing use of nanoparticles in human societies undoubtedly leads to the release of such substances into the environment and ecosystems [3,4]. As it comes to pharmaceutical and chemical sciences, recognizing toxicity of nanoparticles and evaluating the biological effect of nanoparticles are essential [5]. Entered nanoparticles into body cause apoptosis, cell death, damage to the mitochondria, delay in the ovulation, and oxidative stress that is one of the most important factors in the pathogenesis [6,7]. Oxidative stress, occurring during the natural processes utilizing molecular oxygen throughout the body, may cause serious damage to the cell [8]. However, there are several antioxidant mechanisms responsible for counter-balancing oxidative stress. For instance, superoxide dismutase, catalase, and glutathione peroxidase are the key enzymes involved in this system [add literature]. Given that one of the probable mechanisms involved in cell injury stemming from nanoparticles is the increase in leakage of free radicals and oxidative damage, supplying the body with exogenous antioxidant molecules may be considered to be a useful approach. Silymarin is a combination of flavonoids extracted from the sap of seeds of a medicine plant called *Silybum marianum*. For centuries, the seeds of this plant have been used as a useful drug with minimum side effects in the treatment of liver diseases and has been modified to a manufactured drug since 1970 [9]. Three effective substances available in this plant are silybin, silychristine, and silydianine [10]. Silymarin can be absorbed by the intestine and concentrated in the biliary system; furthermore, it has a hepatic circulation [11]. Silymarin retrieves the free radicals, stabilizes the cell membrane and increases intracellular glutathione [12]. Therefore, silymarin may have beneficial effects against nanoparticle induced oxidative stress. In the present study, the primary aim was to investigate whether silymarin may prevent oxidative stress emerged from the application of nanoparticles. To this end, we decided to administer MgO NPs so as to observe the nanoparticle toxicity. As oxidative stress was the main character of this kind of toxicity, malondialdehyde, an index of oxidative stress induced lipid peroxidation [13], was measured in the samples. Additionally, superoxide dismutase, catalase, and glutathione peroxidase were measured as antioxidant markers.

2. MATERIALS AND METHODS

2.1 Animals

32 adult male rats of wistar strain, weighing 350-400g were used for this study. They were housed individually in stainless steel mesh-bottomed cages and were acclimatized before the commence of the experiments at suitable conditions of temperature and light for a period of four weeks.The environmental conditions were set at a temperature of 21-24℃ relative humidity of 55±5% and a 12 h light/dark cycle. This study was carried out according to the guidelines approval by Institutional Animal Ethical Clearance (IAEC). The rats were randomly assigned to four groups, each consist of 8 animals. The first (control) group received 0.5cc distilled water and the second received 0.5cc Magnesium Oxide Nanoparticles at 400 ppm concentration and the third one received 0.5cc Silymarin (sigma company, German) at 200 mg/kg concentration and the fourth group received 0.5cc Magnesium Oxide Nanoparticles at 400 ppm and Silymarin (oral) at 200mg/kg concentration for 4 weeks intraperitoneally. The dose of Silymarin was determined on the basis of present reference and pilot experiment [14]. On four consecutive days at the beginning of the experiment intraperitoneal injections of nanoparticles were performed. The rats did not show any symptoms of toxicity such as modification in fur color, weight loss and any symptoms in the morphology and behavior. Post-treatment tissue levels of Malondialdehyde, Catalase, glutathione peroxidase and the activity of Superoxide dismutase in liver tissue were measured.

2.2 Measurement of Malondialdehyde Levels in Liver

Once finished, the animals were anesthetized with ketamine. Liver was dissected from the body, rinsed with cold saline solution, weighted after dryness promptly, homogenized (10%) subsequently and centrifuged separately with tris buffer for 2 minutes with the homogenizer device

at 5000rpm speed. In order to prevent the elimination of enzymes and proteins, all the above steps were conducted at 4℃ centrifugation, the clear supernatant solution was removed and the bottom sediment was discarded and the clear supernatant solution was used for measurement. Measuring the levels of Malondialdehyde was based on the method of thiobarbituric acid (TBA) reaction and carried out at the boiling point temperature. In this experiment, the MDA or Malondialdehyde-like materials reacted with Thiobarbituric acid and provided a pink color that the maximum absorbance of which was at 532 nm. The reaction was carried out at pH=2-3° and temperate of 90℃ for 15 minutes. After cooling the sample, the absorbance level was observed. To this end, 150 µl of the centrifuged sample were taken and the amount of 1.5cc of Trichloroacetic acid and 1.5 cc of TBARS were added and then we put all the samples and standard tubes with different dilutions into Bnmary hot water for 80 min to facilitate the reaction. The solution was centrifuged at 3000 rpm for 10 min and the absorbance was read in a spectrophotometer at a λ=532 nm. Standard curves were prepared based on the dilution of tetraetoxypropane and the obtained absorbance peaks of sample were adapted on the standard curve [15].

2.3 Measurement of Superoxide Dismutase Enzyme Activity

The SOD activity was measured according to the inhibiting reductions of Nitroblueterazolium by Exanthin-Xanthin oxidase system as the producer of superoxide. In this experiment, the solutions consisted of Xanthine, Xanthine Oxidase were applied in the Potassium Phosphate Buffer and Nitroblueterazolium. The absorbance of each sample was read every 30 second for 5 min. To gain the level of inhibition by SOD enzyme, the obtained data was concluded from the corresponding formula on the basis of set commercial kit. The activity of enzyme was measured by the level of inhibition adapted on the standard curve in terms of u/mg protein.

2.4 Measurement of Catalase Enzyme Activity

The activity of catalase was assayed according to the method of Abie [16]. Briefly 0.01 mml/mml of the Ethanol was added to a definite mass of tissue homogenate and was incubated in ice for half an hour. And Triton X-100, 10% was added to solution with the final concentration of 1%. This solution was used to measure the activity of enzyme. The enzyme reaction was initiated by the addition of 30mM H_2O_2 to extract sample tissue homogenate in Buffer Phosphate Sodium 50mM with pH=70. Then, the absorbance was measured at 40nm within 3 minutes and the enzyme activity was measured in terms of u/mg protein.

2.5 Measurement of Glutathione Peroxidase Enzyme Activity

The activity of GPX was assayed according to the Rotac and et al. method [17] and according to the following reaction:

H2O2+2GS → 2H2O+GSSG (Glutathione Oxide)

Glutathione Peroxidase oxidized Glutathione in tissue homogenates that results in the retrieve of hydrogen peroxide to water simultaneously. This reaction was stopped after 10 minutes by Trichloroacetic acid and remaining glutathione was reactivated by DNTB solution and it led to the formation of colored compounds 420nm that can be measured with a spectrophotometer. Reagent mixture consists of 0.2ml of ethylene diaminetetraacetate (EDTA) 0.8mM, 0.1ml of Sodium azide 10mM, 0.1ml of Hydrogenperoxide 2.5mM, 0.2ml homogenate. The mixture was incubated at 37℃ for 10 min. The reaction was stopped by adding 0.5ml of 10% Trichloroacetic acid and the tubes were centrifuged for 15 min in 2000Rpm. 3ml of Disodium hydrogen 0.8 mM, and 0.1ml DTNB of 0.4% were added to supernatant solution and the resulting color was measured at 420nm accordingly. Glutathione Peroxidase activity was expressed as µ moles of Glutathione oxidized/min/mg protein.

2.6 Apparatus

Electrochemical experiments were conducted by CV using an EA-201 Electro-Analyzer manufactured by Chemilink Systems Co. Morphologic studies and examination of the surface of synthesized MgO NPs were performed by devices consist of a TU-1901 double-beam UV-visible spectrophotometer, a D/Max-RA X-ray diffractometer (XRD) using CuKα radiation, and a JEM-200CX TEM.

2.7 Statistical Analysis

All data were stored in SPSS for operating system of Microsoft .Co (version 19). Group comparisons were carried out using the analysis of variance (one way ANOVA) test. Significant differences between them were assessed by

Bonferroni test. All data were expressed as mean ± standard error of mean (SEM). P-values less than 0.05 were considered to be significant.

3. RESULTS

3.1 Characteristics of UV-VIS Spectroscopy of MgO NPs

This spectroscopy is related to the transition between energy levels (electron states). Such transitions generally take place between the bonding-orbital or non-bonding electron pairs with the anti-bonding orbital; therefore, it is possible to associate wavelength of absorption peaks with various bonds existing in the under study sample [18]. In Fig. 1 a UV-VIS spectroscopy of chemically-synthesized MgO NPsis shown. As shown here (Fig.1), the absorption peak is located in the area of 220-650 nm. The fact that the peak is not sharp indicates that nanoparticles with different sizes are produced in this method. The results of UV-VIS spectroscopy confirmed the data obtained by electron microscopy and proved particular and quantum properties of nanoparticles.

3.2 Electron Microscope Examination of MgO NPs

Though the TEM operates on the same basic principles as the optical microscope, yet in the former (i.e. the TEM) rays come top down. This electron microscope consists of a long column on top of which the source of electron rays is mounted. After transmitting through the specimen, electron rays hit a photographic film or screen (built of fluorescent materials) and create an image [19]. Since some rays do not pass through the sample, black spots are left on the image and therefore electron microscope images are black-and-white. Slices in a TEM are much thinner than those in a light microscope, and staining techniques are also different. In Fig. 2 a TEM image of synthesized MgO NPs is seen. The smaller the size of nanoparticles, the greater their surface area-to-volume ratio; thus, these nanoparticles can play a key role during immobilization processes. According to the results obtained from TEM studies, the size of synthesized MgO NPs was estimated as 70 nm.

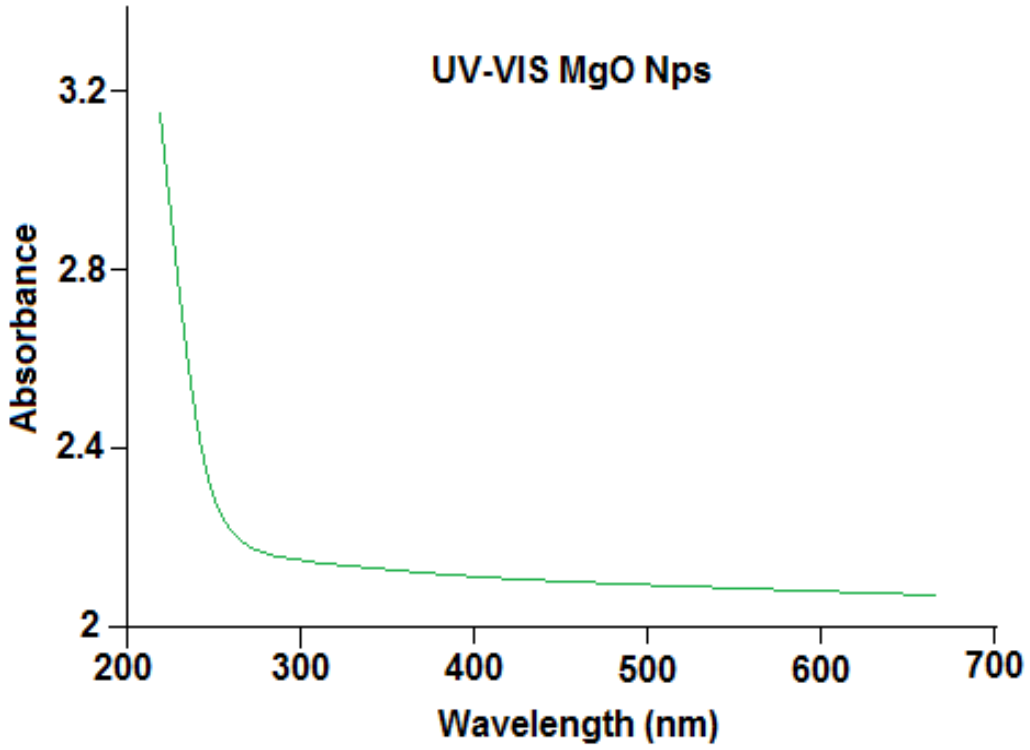

Fig. 1. UV-VIS spectrum of synthesized MgO NPs

3.3 Levels of Malondialdehydein Liver Tissue

By measuring levels of malondialdehyde in different groups (see Table 1) it was found that malondialdehyde in the group receiving the nanoparticles is significantly increased compared with that of in control group (p=0.000). On the other hand, the level in silymarin-treated group was significantly lower as compared with those of either control group (p=0.012) or nanoparticles-treated group (p=0.000).

3.4 Activity of Glutathione Peroxidase in Liver Tissue

By measuring glutathione Peroxidase activity in different groups (see Table 2) it was found that glutathione Peroxidase in the group receiving the nanoparticles is significantly decreased compared with that of in control group (p=0.000). On the other hand, the level in silymarin-treated group was significantly higher when compared with those of either control group (p=0.002) or nanoparticles-treated group (p=0.000).

3.5 Activity of Catalase in Liver Tissue

By measuring Catalase activity in different groups (see Table 3) it was found that Catalase in the group receiving the nanoparticles is significantly decreased compared with that of in control group (p=0.008). On the other, hand the level in silymarin-treated group was significantly higher when compared with those of either control group (p=0.041) or nanoparticles-treated group (p=0.000).

3.6 Activity of Superoxide Dismutase in Liver Tissue

By measuring Superoxide Dismutase activity in different groups (see Table 4) it was found that Superoxide Dismutase in the group receiving the nanoparticles is significantly decreased compared with that of in control group (p=0.001). On the other, hand the level in silymarin-treated group was significantly higher when compared with those of either control group (p=0.029) or nanoparticles-treated group (p=0.001).

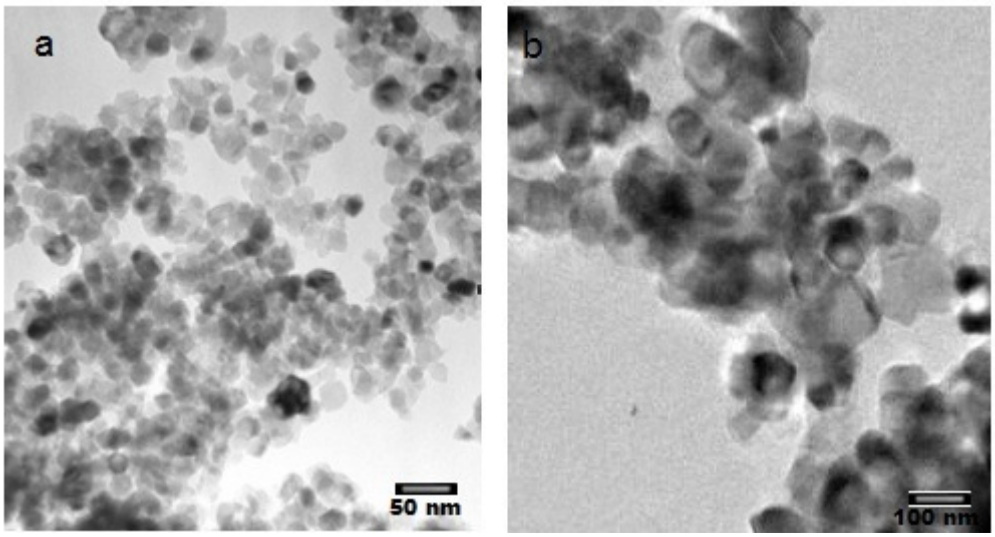

Fig. 2. TEM image of synthesized MgO NPs

Table 1. Levels of malondialdehyde in study groups

Groups	n	Mean	SD	SE	P values
Control	8	3.73	0.72	0.26	
Nanoparticle	8	6.53	1.79	0.64	P=.00***
Silymarin	8	1.9	0.65	0.23	P=0.012*
Nanoparticle+Silymarin	8	3.3	0.67	0.24	P=.00###

**: compared with the control group, #: compared with the nanoparticle-treated group, P value: significant less than 0.05, *: p<0.05, ***: p<0.001, ###: p<0.001*

Table 2. Levels of GPX in study groups

Groups	n	Mean	SD	SE	P values
Control	8	83.3	1.91	0.68	
Nanoparticle	8	74.82	3.85	1.36	P=.000***
Silymarin	8	89.62	3.02	1.068	P=.012*
Nanoparticle+Silymarin	8	85.73	3.096	1.095	P=.000 ###

**: compared with the control group, #: compared with the nanoparticle-treated group, P value: significant less than 0.05, *: p<0.05, ***: p<0.001, ###: p<0.001*

Table 3. Levels of CAT in study groups

Groups	n	Mean	SD	SE	P values
Control	8	40.25	4.37	1.55	
Nanoparticle	8	32.35	5.51	1.95	P=0.008**
Silymarin	8	46.75	2.68	0.95	P=0.041*
Nanoparticle+Silymarin	8	46.375	4.78	1.69	P=.000 ###

**: compared with the control group, #: compared with the nanoparticle-treated group, P value: significant less than 0.05, *: p<0.05, ***: p<0.001, ###: p<0.001*

Table 4. Levels of SOD in study groups

Groups	n	Mean	SD	SE	P values
Control	8	5.04	1.27	0.45	
Nanoparticle	8	2.54	0.59	0.21	P=0.001***
Silymarin	8	6.8	1.72	0.61	P=0.029*
Nanoparticle+Silymarin	8	5.05	0.59	0.21	P=0.001###

**: compared with the control group, #: compared with the nanoparticle-treated group, P value: significant less than 0.05, *: p<0.05, ***: p<0.001, ###: p<0.001*

4. DISCUSSION

This study showed that in the rats exposed to nanoparticles, more free radicals are released in the liver and the antioxidant enzymes decline in a way the enzyme MDA increases and the GPX, CAT, SOD enzymes would reduce, but the rats exposed to silymarin were backfired. The oxidative stress (enzyme MDA) reduced in the mice that were treated with nanoparticles once and the antioxidants enzymes (SOD, GPX, CAT) were increased exposing to silymarin. It is concluded that silymarin has the antioxidant properties and decreases the GPX activity in contrast with Magnesium nanoparticles and this result may be due to the antioxidant properties of Silymarin. Previous studies have shown that in nanoparticles mode, generally the oxidative stress increases due to the preparation of free oxygen radicals and the antioxidant defense system shows a decline in function. These changes are responsible for tissue damages partially [20] as found in the present study. The induction of antioxidant enzymes is a main approach to protect cells against a variety of internal and external toxic compounds such as oxygen free radicals [21]. A reduction in the activity of Super Oxide Dismutase in mice exposed to nanoparticles were observed in this study which may be resulted from an increase in Lipid Peroxidation as well as that of production in oxygen free radicals, which is consistent with the results of previous studies [22] Gold nanoparticles induce the production of oxidative stress in rats with diabetes mellitus within 24 hours [23]. Studies have shown a significant Peroxidation in mice livers treated with nanoparticles. Liver and spleen are two main target metabolic organs for nanoparticles [24]and this approved that nanoparticles with the diameter less than 5.5nm could be excrete from kidney [25]. If the particles are larger than the renal filtration, they don't excrete via urine. So, they dispose from the body by the reticuloendothelial system and concentrate in the liver [24] However, the treatment with silymarin could improve the process significantly. In this regard, it was shown that some flavonoids, such as silymarin can increase the antioxidants activities in the body and intensify the antioxidant enzymes activities [26] as well as causing decrease in Lipid Peroxidation [27]. silymarin causes inhibition in oxidative stress by collecting the Oxygen free radicals such as Hydroxyl Anion radicals Phenoxy and Hypochlor acid in various types, such as Platelets and Fibroblasts, liver

Microsomes and Mitochondria and have the ability to protect the neurons against oxidative stress by preventing the Lipid Peroxidation and modification of glutathione [28]. Itaffects the cell membrane properties by accumulation of free radicals and iron buffering [29] .

5. CONCLUSION

This study showed that four-week silymarin administration at the dose of 200mg/kg/w.b of rats has an antioxidant effect and can reduce some symptoms of the oxidant stress in the animal liver tissue; so, the effect of MgONPs is to promote free radicals and leads to tissue damages.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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