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The effect of MgO@FO nanoparticles on liver enzymes in male mice injected with Thioacetamide

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ABSTRACT: Magnesium oxide nanoparticles are characterized with a wide variety of applications and are massproduced throughout the world. However, questions remain regarding their safety. In this study, the hepatoprotective effects of fig and olive hydroethanolic extract in Quranic ratios of 1: 7 in the prevention of thioacetamide-induced liver damage in male Syrian mice were investigated. In addition, hematology, biochemistry, and histopathology of the rats are examined at various concentrations (75,150,300 mg / kg) over 28-days period. In this experimental study, 36 male rats were divided into 6 groups of 6. Control without any drug treatment, the control group treated with thioacetamide 100 mg / kg, the third group treated with 300 mg / kg metformin, and groups 4, 5 and 6 with coated concentrations of 75,150,300 mg / kg, were gavaged every other day for 28 days. At the end of the experiment and the mean activity of ALT, AST and ALP enzymes was compared with the control group. The results of one-way analysis of variance (ANOVA) showed a significant increase in serum SGOT level in thioacetamide + metformin and thiostamide groups compared to other groups (P <0.001). Thioacetamide + metformin and thioacetamide were seen compared to other groups (P <0.001). A significant increase in ALP enzyme activity was observed in all groups compared to the control group receiving 150mg / kg coated nanoparticles (P <0.01). The combination of hydroathanolic extract of fig and olive fruit with Quranic ratios may modulate liver enzymes.

Keywords: *Enzymes; Evaluation; Hydroethanolic Extract; Magnesium Oxide; Nanoparticles*

INTRODUCTION

Nanoparticles are miniscule materials (with at least one dimension less than 100 nm) with unique properties, which make them suitable for novel applications. These attributes make them very attractive for commercial and medical developments. In recent years, the break-throughs in nanotechnology have been accompanied with inorganic and organic nano-sized particles with growing applications to be used as modifier in industrial, medicine, therapeutics, synthetic textiles, and food packaging. Moreover, nanoparticles are expected to play a crucial role in water purification. Now, the rapid increase in world's population and shortage in fresh water demand appropriate, cost-effective, and rapid wastewater treatment techniques. By nanotechnology, water and wastewater can be treated not only to deal with main challenges of present treatment technology, but also to provide new treatment potentials, which in turn allows economic exploitations of unconventional water sources as a water supply. As Sawai *et al.* pointed

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out, the moisture absorptions on the MgO nanoparticle surfaces, which forms a thin water layer around the particles, is a possible antibacterial mechanism. The local pH of the mentioned water layer can be greater than its equilibrium value under solution sets. When the nanoparticles are faced with bacteria, the high pH in that water layer could damage the membrane, resulting in cells death [1-6]. Given the unique properties of these particles, nanotechnology is also being applied in medical sciences. Recently, the initiative methods as per development of nanoparticle drugs have emerged in cancer treatment. Such particles offer controlled drug delivery; enhance permeability, and tumor specific targeting. In addition, nano-drugs cope with carcinogenic cells, enter them in an easy manner and, at the same time, have little side impacts on normal cells. The most important characteristics of these drugs are particle size, molecular weight, pH, ionic strength, monomer concentration, and surface charge among others. At the same time, potentially nanoparticles have great contribution in diagnosis and imaging of brain tumors.

In medicine, MgO is used for the alleviation of heartburn, stomach sore, and for bone regeneration. Nowadays, MgO nanoparticles are applied in tumor inhibition and additionally, have remarkable potential as an antimicrobial agent. Other experimental results revealed the possible utility of MgO nanoparticles in the treatment of cancer, including Nanocryosurgery and hyperthermia. The applications of MgO nanoparticles and also the issues on its feasible toxicity are increasing. Unfortunately, there is a paucity of knowledge about the effect of the prolonged exposure to nanoparticles on human health and the environment. Before their large scale production and application in diverse fields, the impact of nanoparticles on health and environment merits more assessment. Hence, estimating the cost/benefits ratio for utilizing nanoparticles in technological or medical procedures is of great importance. The nanomaterial toxicity such as nanoparticles, quantum dots, nanotubes, and nanowires has been declared in the past few years. In this respect, various studies dealt with the toxicity of MgO nanoparticles. Lai *et al.* showed that treatment of human astrocytoma (astrocytes-like) U87 cells with MgO nanoparticles for 48 h did not significantly decrease their survival until the concentrations were higher than 50 $\mu g.mL^{-1}$.

MATERIALS AND METHODS

Synthesis, coating and evaluation of properties

Wet chemical method was used to synthesize MgO nanoparticles. First, three solutions of mercaptoethanol (ME), sodium hydroxide (NaOH) and magnesium sulfate (all from Merck) were prepared in distilled water and stirred vigorously. The magnesium sulfate solution was then poured into a balloon container. A diluted inhibitor drop was then added to the same balloon every 4 seconds. Finally, sodium hydroxide was added to the balloon in the same way until the solution gradually turned white until a white solution was obtained. To extract any impurities, the final solution is washed several times with deionized water and after washing, the solution is poured into a human and placed in an oven (at a temperature of 70 ° C for 48 hours to dry. After 48 hours, nanoparticles we take the dried material out of the oven, then pour it on a clean surface and powder it with a tool, pour the powdered material into a jar and put it in the oven and set the oven temperature to 700 ° C. And put the material in the oven for 3 hours to dry completely (Note: the three hours we have to consider for drying should be calculated from the time when the oven temperature has reached 700 degrees Celsius). To prepare 70% hydroethanolic extract, you must first mix 70 cc of ethanol with 30 cc of distilled water in an Erlenmeyer flask and then carefully weigh 50 g of dry plant powder (6.5 g of fig powder + 43.5 g of olive powder) carefully. It is weighed and poured on a mixture of ethanol and distilled water. The lid is tightly closed and then placed on a shaker (Rotator model 2002 from Behdad-Iran) for 48 hours to mix the ingredients thoroughly. After 48 hours, remove the mixture from the shaker and then pass it through filter paper. Fig and olive extract were used to coat magnesium oxide nanoparticles. Pour 0.6 g of nanoparticles in 100 cc of distilled water and dissolve. Then pour the dissolved nanoparticles into the balloon and connect the suction tube to the balloon. Pour 100 cc of the filtered extract into the soxhlet and open the milk so that a drop of the

extract is added to the nanoparticles every 4 seconds. After this, add some distilled water to the solution and wash it several times to remove excess extracts. After washing, pour the solution into the plate and place it at a temperature of 40 ° C until the solution evaporates and the powder is obtained. After the solutions dry, the extracts are shaved from the bottom of the plates and collected.

Grouping and treatment of laboratory animals

In this study, male Wistar rats weighing 200 + -20 g were used. The animals were randomly grouped and kept under standard conditions of 12 hours of light and 12 hours of darkness with a temperature of 2.23 °C. During the keeping period, the animals had free access to sufficient water and food. Hydroethanolic extraction method was used to adapt and bring the extract closer to the traditional medicine system and the Quranic subject. Dried powder was used to prepare doses of 75, 150 and 300 mg / kg body weight in mice. The animals were randomly divided into groups of 6. All groups except the control group received thioacetamide at a concentration of 100 mg / kg after 28 days of daily gavage to mice on day 29. Intraperitoneal injection of thioacetamide into mice was performed.

The first group was healthy controls who received only water and food. The second control group received thioacetamide injection, which received thioacetamide (liver toxin) only on the 29th day without medication. The third group received metformin at a concentration of 300mg/kg. The treatment groups 4, 5 and 6 received the extracts of fig plants + synthesized magnesium nanoparticles by gavage with doses of 75, 150, 300 mg / kg of buddha, respectively. The important point is that all groups received drugs and extracts by gavage one day out of 0.5. At the end of the 28th day after a day of rest and on the 29th day, all groups except the control group received thiostamide in the amount of 0.5 cc by intraperitoneal injection. 24 hours after the last treatment, at the end of the experiment after anesthesia of mice, the collected blood was centrifuged for 4 minutes with 4000 cycles and their serum was removed. Blood serum taken from the heart of mice was used after collection for liver enzyme activity, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were measured. The results of this study were analyzed by SPSS software (Version 22) and one-way analysis of variance (ANOVA).

Measurement of liver enzymes

After blood sampling and isolation of sera, the sera of mice were referred to Kashif Laboratory located in Falavarjan to perform the necessary tests to evaluate and evaluate liver enzymes on sera with a laboratory kit.

Statistical analysis

In this study, after preparing the information of blood sampling results in Excel file, data analysis was performed using the general difference of meaning 1 of SPSS software version 22. Therefore, first, using ANOVA method between different groups was determined, using Duncan test, the differences between the treatment groups and each group were examined separately for the factors that showed differences. One-way analysis of variance was used to compare the means of the groups. Then the mean activity of enzymes in different groups was compared with each other and with the control group. Significance levels of 1% and 5% were considered.

RESULTS

The results of ALP activity are shown in Fig. 1A. In this category, the best group of coated nanoparticles was at a dose of 150mg/kg because ALP activity was significantly reduced compared to coated nanoparticles at doses of 75 and 300mg/kg. Comparison of the average ALP activity in different groups showed that the activity ALP was increased in the group injected with thioacetamide compared to the control. This increase was statistically significant ($p = 0.001$). Mean ALP activity in metformin which also increased compared to thioacetamide group ($p = 0.001$). The best group of coated nanoparticles + thiostamide is concentrated (150mg/kg) which significantly reduces the average ALP activity and then the group of coated nanoparticles with concentration (75 mg/kg) is the best group. Also, different concentrations of nanoparticles performed better than metformin and reduced

the average ALP activity by the control group.

The results of AST (SGOT) activity are shown in Fig. 1B. Comparison of the mean of SGOT activity in different groups showed that the amount of SGOT activity in the group injected with thiostamide increased compared to the control group, which was statistically significant (p = 0.000). The mean SGOT activity in metformin was not statistically significant compared to the thiostamide group. The best group is nano 75mg/kg, which significantly reduced the mean SGOT activity, followed by the coated nanoparticles group (75 mg/kg + thiostamide) at the best dose are golden. However, all nano-concentrations performed better than metformin and reduced the mean SGOT activity in the control group. Also, one-way analysis of variance with Duncan test showed a significant increase in the thiostamide + metformin and thiostamide groups compared to the other groups (P <0.001).

The results of AST activity (SGPT) are shown in Fig. 1C. Changes in serum ALT enzyme generally go hand in hand with AST enzyme. Therefore, one of the reasons for the increase in serum levels of these enzymes may be a change in the permeability of the plasma membrane of liver cells or cellular damage resulting from exposure to nanoparticles. The results of ALT activity in different groups showed that the level of SGPT activity in the group injected with thiostamide increased compared to the control group, which was statistically significant (p = 0.000). The mean SGPT activity in metformin was not statistically significant compared to thiostamide group. The best group of nanoparticles is 75mg/kg, which significantly reduces the average SGPT activity, followed by the coated nanoparticles (300mg/kg + thiostamide) of the best available doses. However, all nano-groups performed better than metformin and reduced the mean SGPT activity by the control group.

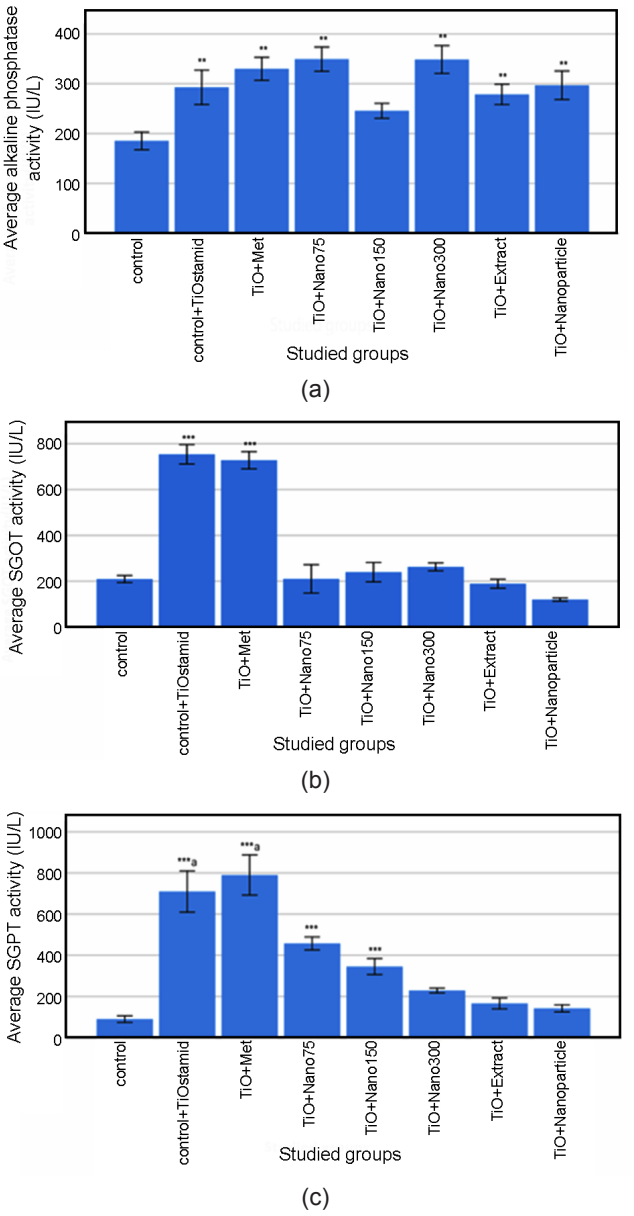

Fig. 1. Biochemical results for rats treated with MgO nanoparticles 28 days after intraperitoneal injection at different concentrations (75-300 mg/kg). These results indicate the mean and standard division: (a) alkaline phosphatase. (b) Aspartate aminotransferase. (c) Alanine aminotransferase. Abbreviations: AST, aspartate transaminase. ALT, alanine transaminase; ALP, alkaline phosphatase.

DISCUSSION AND CONCLUSIONS

The biochemical effects of different doses of MgO@FO coated nanoparticles were investigated, as shown in Table 1 and Fig. 1, including aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Olives are used in the treatment of heart and liver diseases and lowering blood pressure. The oleuroposide in olive leaves and fruits are responsible for the hypoglycemic activity of the plant. Olives have an inhibitory effect on the angiotensin-converting enzyme. Oleuropein, oleacin and phenolic compounds in olives prevent the oxidation of low-density lipoprotein (LDL) while lowering total and free cholesterol. Therefore, olives have anti-atherosclerotic properties.

Table 1. Comparison of liver enzymes in control and control groups with thioacetamide injection.

ALT enzyme (mg / kg)	AST enzyme (mg / kg)	ALP enzyme (mg / kg)	group
89.8000±36.25190	209.4000±34.33366	185.3333± 43.32051	Control
709.7500±200.64126	754.0000±73.00000	292.8333±84.35500	Control by injection of thioacetamide (100mg/kg)
790.3333±169.16658	728.0000±65.19969	330.0000±51.58488	T + met (300mg/kg)
457.5000±44.54773	210.0000±87.68124	349.4000±54.52339	T + Coated Nanoparticles (75 mg /kg)
345.0000±55.15433	239.5000±103.37263	245.6667±36.70241	T + Coated Nanoparticles (150 mg /kg)
228.5000±16.26346	262.5000±24.74874	348.6667±48.42864	T + Coated Nanoparticles (300 mg /kg)

Values are checked as mean ± standard error for 6 animals.

The hydroxytyrosol present in olive extract reduces the formation of large clots through platelet aggregation as well as reduced production of thromboxane A2. While oleuropein, tyrosol and caffeic acid inhibit the production of leukotriene B4 at the 5-lipoxygenase level. Olives have anti-thrombotic properties. Squalene in olive oil also prevents the synthesis of cholesterol. The liver is involved in food processing, excretion of toxins and production of bile acids, which is due to its unique double blood circulation. The liver has a variety of functions. This organ plays a central role in nutrition and metabolism of vitamins. The liver is responsible for the synthesis of vital proteins such as albumin, coagulation factors, apoproteins, etc. and plays an important role in the function of the immune system by having macrophage and Kupffer cells. It pollutes the environment and chemical drugs [8-10]. Olives contain antioxidant compounds, possibly by direct purification of reactive oxygen species, increase the ability of oxygen radicals to be absorbed into cells, reduce lipid peroxidation, and inhibit mutations by environmental toxins, KB, and the activity of mRH and binchenogen mRH. 2. COX and interleukins exert their anti-inflammatory effects, reducing the activity of serum ALT and AST enzymes. Olive fruit contains potassium, which is one of the essential ions; it must be supplied from food sources. Research has shown that this bone can be linked to cancer and its prevention. Diets high in sodium and potassium pabin can cause the growth of tumor cells because they change the normal pH as well as the water balance in human cells.

One of the biochemical markers for the assessment of liver disorders is the enzyme alkaline phosphatase (ALP), which is mainly attached to the cell membrane. The most common causes of increased alkaline phosphatase activity are bile duct damage and destruction of liver cells. Also, an increase in the alkaline phosphatase enzyme up to three times its normal level in the blood is an indicator of the occurrence of liver problems. Therefore, this enzyme is another evidence for the confirmation of liver damage. Figs are one of the fruits that have the highest sources of calcium and fiber along with copper, manganese, magnesium, potassium, calcium and vitamin K and are rich in flavonoids, polyphenols such as gallic acid and rutin. Figs contain antioxidants that repair damage to cells and tissues. Apart from its antioxidant properties, figs also have antimicrobial effects [10-15].

The thioacetamide used also damaged liver cells. On the other hand, the mechanism of thioacetamide damage to the liver is due to oxidative stress. Thioacetamide is a chemical that is used to induce cirrhosis of the liver and acute hepatic encephalopathy. Thioacetamide is metabolized by enzymes in the cytochrome P450 detoxification system upon entry into the body. Metabolism of thioacetamide produces other thioacetamide S-oxidometabolites. Finally, thioacetamide S-oxide is an intermediate compound in the oxidation steps of thioacetamide by monooxygenases with dif-

ferent functions that cause oxidative stress in liver cells. Also, thioacetamide in high doses causes necrosis and apoptosis of liver cells. On the other hand, free radicals from thioacetamide attack the membranes of liver cells and cause lipid peroxidation, which reduces the fluidity of the membrane and, as a result of its permeability, releases substances and proteins, including albumin, into the serum. From the present study, it can be concluded that thioacetamide at a concentration of 100 kg / mg also had a significant increase in the activity of liver enzymes. As a result, thioacetamide is probably from Increases serum levels of transaminases and damages liver tissue structure by causing oxidative stress. Thioacetamide is used in laboratory organisms to induce liver cirrhosis and acute hepatic encephalopathy; the results of a study showed that thioacetamide causes cell death, necrosis and apoptosis in liver cells. Thioacetamide is a liver toxin and by acting on the synthesis of DNA, RNA, protein and glutathione content, it causes structural and functional changes in the liver organ.

The results of this study showed that magnesium oxide nanoparticles coated with hydroethanolic extract of fig and olive caused significant changes in the activity of AST, ALT and ALP enzymes. The results of the present study show that the level of aspartate aminotransferase enzyme was significantly increased in mice injected with thioacetamide (P = 0.000). There was no statistically significant difference with thioacetamide group, so it can be concluded that metformin did not have much effect on liver protection. We must also say that all nano-groups performed better than metformin and were able to reduce the activity of SGOT enzyme in the control group. Magnesium oxide nanoparticles coated with hydroathanolic extract of fig and olive oil at concentrations of 75 kg / mg body weight caused a significant decrease in serum aspartate aminotransferase activity in male mice compared to control mice. Also, aspartate aminotransferase in thioacetamide and metformin groups had a significant increase compared to other groups (P <0.001). In the alanine aminotransferase enzyme group, the results of the present study show that the level of alanine aminotransferase enzyme significantly increased in the mice of the second group, i.e. injected with thioacetamide (P = 0.000). Also, the mean activity of alanine aminotransferase enzyme in metformin, which is a therapeutic drug for the liver, was not statistically significant compared to the thioacetamide group. Also, nanocomposites with a concentration of 300 mg / kg body weight were the best doses affecting the activity of alanine aminotransferase, which significantly reduced the activity of serum alanine aminotransferase in male mice compared to mice injected, created with thioacetamide. Also, a significant increase was observed in the 150 and 75 mg / kg groups of thiostamide + metformin and thioacetamide compared to the other groups (P <0.001). The results of the present study showed that the activity of alkaline phosphatase enzyme was significantly increased in mice injected with thioacetamide (P = 0.001). Thiostamide increased statistically (P = 0.001). The best effective doses were in the groups of coated nanoparticles with a concentration of 150 kg / mg. In the study of ALP enzyme activity, a significant decrease in the mean activity of ALP enzyme was observed at a dose of 150 kg / mg of coated nanoparticles compared to the group injected with thiostamide. As a result, it can be said that the best doses of gold in the activity of liver enzymes in mice were usually 75 and 150 kg / mg doses in coated nanoparticles, which could reduce the activity of liver enzymes to some extent, and could even work better than metformin. And metformin had little effect on the reduction of liver enzymes. Due to the physiological similarity of mice with the human body, magnesium oxide nanoparticles coated with fig and olive extracts in the above effective doses could be suggested as a supplement for the treatment of hepatitis in humans.

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