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Antioxidant effect of *Peganum harmala* L on toxicity caused by silver nanoparticles in mice liver

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Key words: *Peganum harmala* L (Ph), Nanosilver particles (Ns), Catalase, Glutathione peroxidase, Malondialdehyde, Liver tissue.

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Abstract

The research objective is to examine the effect of Ph (*Peganum harmala* L) ethanolic extract on liver tissue, malondialdehyde density, catalase and glutathione peroxidase activity of male mice, treated by nanosilver particles. The research was conducted on adult male mice of albino race (25-30gr). The animals were divided into four groups. Group 1, as control, Group 2 were treated by nanosilver particles, Group 3 received 20mg/kg/day of oral Ph ethanolic extract for 30 days. Group 4 is the composition of the Group 2 and 3. The activity rate of catalase (CAT) serum and glutathione peroxidase (Gpx) of red blood cells, density of malondialdehyde (MDA) serum were determined .The collected sections of liver tissue were stained with hematoxylin and eosine in order to be studied. The liver tissue of mice treated with nanosilver particles and Ph ethanolic extract, was normal as it was in control group. Malondialdehyde showed significant decrease, and catalase and glutathione peroxidase activity increased significantly ($p<0/001$) in comparison with group 2 (treated with nanosilver particles 500NS). The results of this research show that antioxidant property of Ph ethanolic extract prevents liver damage caused by free radicals resulting from silver nanoparticles. It can even be introduced as a reducer of free radicals induced by silver nanoparticle.

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Introduction

Increasing development of nanotechnology has attracted the attention of many researchers and experts, but at the same time there are growing apprehension and worries about its environmental dangers. Technology of nanosilver particles is an applied branch of nanotechnology. Because of pesticidal and anticeptic properties of silver, it is widely produced and used in nanodimensions. This shiny white metal with atomic weight of 47 is a very conductive, flexible rare element found in nature. Pure silver is an element with the highest level of toxicity and the least contact resistance in comparison with other metals (Nordberg *et al.*, 2011).Silver has been used as an antibacterial substance for a long time(Hussain *et al.*, 2005). The most common effects of long-term contact with silver are irreversible defined skin and ocular pigmentation (agyria and argyriosis, respectively). Nanosilver particles may affect different cells and have toxic effects. Sriram and his colleagues (2010) showed that silver nanoparticles can activate caspase mitochondrial enzymes, particularly caspase3, in cancerous lymphatic cells and bring about programmed cell death apoptosis (Sriram *et al.*, 2010). In fact, catalytic mechanism of silver nanoparticles causes O2 oxidation and its transformation in to free radicals of O2.-, or water hydrolysis and production of OH-. As it is known, free radicals are very powerful reactives and have a strong tendency to receive electron to make their electrons paired. These processes damage other molecules or disrupt their functions. Actually, lipids are the most important molecules attacked by free radicals. The process of peroxidation eventually results in cell death(Jeddi and Ahmadi, 2008). The final product of lipids peroxidation is malondialdehyde (MDA). There is now a possibility that occurrence of apoptosis will be similar to the effects of silver nanoparticles on liver cells (Pryor *et al.*, 1976). In this case, resultant radicals are harmful to the body and dangerous for one's health. Today silver nanoparticles as a deterrent activity against microorganisms have been used successfully in various industries and mortality studies of HIV-1 is influenced by the material(Vega-Lopez *et al.*, 2004)). A recent report by Hussain and colleagues(2005) on the mitochondrial toxicity of silver nanoparticles showed that these nanoparticles after entering the body and liver uptake, via ROS production and reduced glutathione, caused abnormalities in liver function(Hussain *et al.*, 2005). Hansen and Nagley in 2003 showed that free radicals produced by the catalytic mechanism of silver nanoparticles causes cells aging or apoptosis through the process of lipid peroxidation.(Hansen and Nagley, 2003).There are antioxidant molecules which maintain oxidative hemostasis and protect against oxidative stress. These molecules either prevent the production of reactive oxygen species, or neutralize them, or stop them from having any effect (Hori *et al.*, 2002). Glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are some of antioxidant enzymes (Sikka, 1996). An important feature of these enzymes is that they can be induced under oxidative stress condition (Mates *et al.*, 1999). Glutathione peroxidase and catalase enzymes transform hydrogen peroxide into water and oxygen(Oruc and Usta, 2007). Study of the compounds of herbal origin is an interesting branch of medical sciences (Mates *et al.*, 1999). Many of these compounds have preventive effects and can most probably be used to control certain diseases in yome populations (Yuspa, 2000). Nowadays, fruit and vegetable consumption is increasing because of their protective properties against cancer, liver and cardiovascular diseases(Pyo *et al.*, 2004). Some herbal raw extracts used in traditional medicine are rich sources of compounds with preventive and protective properties useful for liver(Pyo *et al.*, 2004). *Peganum harmala*(Ph) is a plant with antioxidant and antimutagenic properties(Germano *et al.*, 1999). Antioxidants are substances with the ability to fight against oxidative stress(Moura *et al.*, 2007). Some of the antioxidants found in *Peganum harmala* L. are phenols, polyphenols (flavonoids) and tocopherol(Hansen and Nagley, 2003; Bratton *et al.*, 2000). These compounds may reduce oxidative stress resulting from the catalytic activity of nanoparticles. However, in a study, the seed extract of *Peganum harmala* orally were used on axis of Pituitary-Thyroid for 17 days and toxic dose was

determined 300mg/kg(Hosseini *et al.*, 2010). Considering that silver nanoparticles are widely used in different fields and due to the fact that there is very little knowledge about antioxidant effect of *Peganum harmala* L. and its ability to destroy free radicals resulting from silver nanoparticles, this research has been designed to study the effects of Ph on the transformation of antioxidant enzymes and the structure of mice liver tissue exposed to silver nanoparticles. So that, after treatment of animals with silver nanoparticles, the antioxidant effect of *Peganum harmala* extract orally examined.

Materials and methods

Animals

This survey has been carried out experimentally on 32 male mice of albino race. They were bought from pasture Institude in Tehran and were kept in the animal room of Islamic Azad university of Falavarjan at a suitable laboratory temperature (22±2 degrees of Celsius) for a month in order to be prepared for the experiments. They weighed 25-30 gr.

The method of preparing ethamolic extract

Peganum harmala L.. seeds were gathered from desert around Isfahan. The species was identified and the result was confirmed in the herbarium of Falavarjan Islamic Azad University. Then, 200gr. of the seeds were washed and powdered in an electric grinder after being dried in a dark place. Thirty-two grams of Ph powder with ethanol 100% were extracted by sox let extractor at 50℃ in 48 hours. Then, it was draind in a rotary device, and the obtained solid powder was diluted b double distilled water. A dose of 20mg/kg/day of this solution was fed to the mice for 30 days. The given dose of 20mg/kg/day for 30 day was considered according to the average percentage of the mice's weights and toxicity range of the solution.

Experimental groups

There were four experimental groups, each consisted of 8 mice. Group1 as control group received 1ml of distilled water through intra-peritoneal injection for 3 successive days. Group2(500 Ns) received 0.5 ml of silver nanoparticles with density of 500 ppm through intra-peritoneal injection for 3 successive days. Group3(Ph) received 0.5 ml of distilled water, as group1 did, and also 20mg/kg/day of Ph ethanolic extract orally for 30 days. Group4(Ns.Ph) received 0.5ml of silver nanoparticles with density of 500ppm as group2(500 Ns) did, and also 20mg/kg/day of Ph ethanolic extract orally for 30 days. It is noteworthy that all of injections in all groups were repeated for 3 successive days before 30 days of treatment. Furthermore, group3(Ph) and group4(Ns.Ph) were treated with Ph ethanolic extract for 30 successive days, after the third injection of distilled water and silver nanoparticles, respectively. In order to eliminate shock-induced symptoms, control group received 0.5ml of distilled water through injection. After 30 days blood sample was taken from their heart. To assess the activity of catalase serum, Abei method-based on the rate of hydrogen peroxide breakdown at the wavelength of 20nm was applied (Aebi, 1984). The activity of glutathione peroxidase of red blood cells was assessed indirectly by reaction paired with glutathione reductase(GR). Reduction of glutathione oxide resulting from glutathione peroxidase reaction occurred by means of NADPH at the presence of GR. In this reaction, NADPH oxidation to $NADP^+$ decreased absorbtion at the wavelength of 340nm that corresponded to GPx activity (Paglia and Valentine, 1967). The amount of MDA serum was measured based on Satoh method(Satoh, 1978). It should be mentioned that the spherical silver nanoparticles with average diameter of 10nanometres were provided by Nanonasb Pars Company.

Microscopic studies

For microscopic studies, separated liver tissue was put in formalin10%, as a buffer, in order to be preserved. Sections obtained from the liver tissue were stained with hematoxylin and eosine (Luna , 1968). The structure of the liver tissue was studied by the light microscope with a 100-dioptre lens. Some Photomicrographs of damaged areas were prepared.

Statistical Analysis

One way ANOVA and Dunkan test were used to compare changes in the amount of MDA, CAT serum and GPx of red blood cells. Significance level of variance was considered p<0.0001. samples were randomly selected and divided in to 4 groups. Within each group the experiments were repeated on 8 mice. Moreover, SPSS 15 software was used to assess data statistically and to draw the graphs.

Results

Study of malondialdehyde levels changes in all groups

MDA average concentration in control group was 22.41±1.63 nmol/ml thirty days after the experiment, while if as 77.12±5.16 nmol/ml in group 2(500 Ns) after 30 days, showing a significant increase(p=0.00). MDA average concentration in group3(Ph) was 34.25± 5.33 nmol/ml after 30 days, showing a significant increase compared with control group. In group4(Ns.Ph) it was 40.41±1.041 nmol/ml showing a significant increase(F=37.852,P<0.001) in comparison with control group (Fig.1).

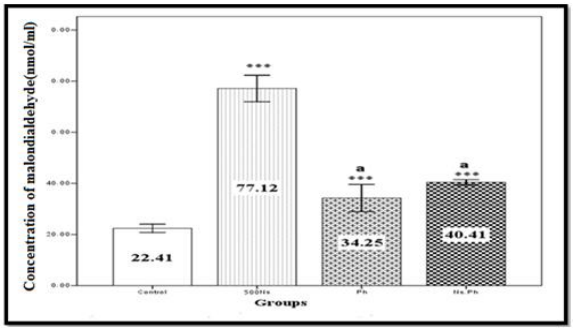

Fig. 1. Comparison of MDA concentration between control group and each of the treatment groups. Each column shows mean ±standard error (Mean ±SE). the difference between control group and every other group, marked with 3 asterisks at the tap of the column, is significant(p<0.001). Mean of each column has been written on it. There is no significant difference between the two groups marked with letter a. There is a significant difference between group2 and 3 also between group2 and 4(p<0.001).

Fig.1 shows that MDA concentration in groups2,3 and 4(Ns, Ph and Ns Ph respectively) is significantly higher than that in control group(p=0.00). However, MDA concentration in groups3 and 4(Ph and Ns.Ph respectively) is significantly lower than that in group 2(500 Ns).

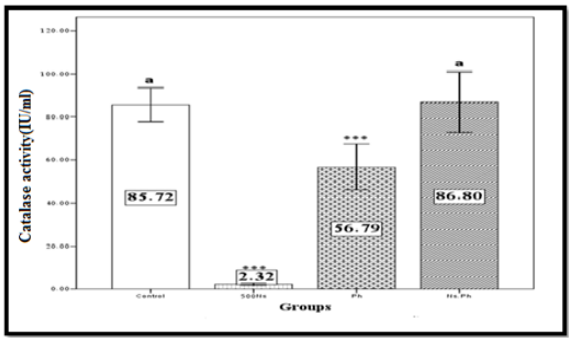

Fig. 2. Comparison of catalase activity between control group and each of the treatment groups. Each column shows mean ± standard error (Mean ±SE). The difference between control group and each of the two groups, marked with 3 asterisk at the top of the column is significant (p<0.001). Mean of each column has been written on it. The two groups marked with letter a do not show any significant difference. There is a significant difference between group2 and group3, group2 and group4, and also group3 and group4(p<0.001).

Study of catalase levels changes in all groups

Thirty days after the experiment, the average rate of CAT activity in group2(500 Ns) was 2.32±0.49 IU/ml which showed significant decrease in comparison with that in control(p=0.00). The average rate of CAT activity in group3(Ph) was 56.79±10.63 IU/ml, after 30 days, showing a significant decrease compared with control group. In group 4 (Ns.Ph), the average rate of CAT activity was 86.80±14.14 IU/ml, which was similar to that in control group and showed no significant difference (P<0.001, F=16.757)(Fig. 2).

Fig. 2 shows that catalase activity rate in groups2 and 3(500 Ns. and Ph respectively) decreases significantly compared with that in control group(P=0.00). However, there is no significant difference between control group and group4 (control and Ns.Ph respectively).

Study of changes at glutathione peroxidase levels in all groups

Thirty days after the experiment the average rate of GPx activity in control group was 9.98 ±0.90 U/gHb,

while it was 2.93±0.60 U/gHb in group2(500 Ns).Showing a significant decrease compared with that in control grop (p=0.00).The average rate of GPx activity in group3(Ph) was 6.59 ±1.61 U/gHb after 30 days, showing a significant decrease compared with control group. In group4(Ns.Ph), the average rate of GPx activity was 8.36±0.57 ,which was similar to that in control group and showed no significant difference (P<0.001, F=8.897)(Fig. 3).

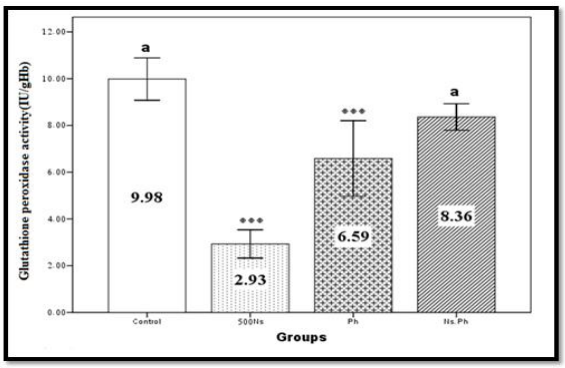

Fig. 3. Comparison of GPx activity between control group and each of the treatment groups. Each column shows mean ±standard error (Mean ±SE). The difference between control group and each of the two groups, marked with 3 asterisk at the top of the column is significant (p<0.001). Mean of each column has been written on it. The two groups marked with letter a do not show any significant difference. There is a significant difference between group 2 and group3, group2 and group4, and also group3 and group4 (p<0.001).

Histopathological study of liver

Microscopic study of the samples of group1(control group) showed that the structure of liver tissue was completely normal(Fig. 1). In the sample tissue of group 2(500 Ns) atrophy of hepatocyte cords occurred and the nucleus of hepatocyte was unclear in most cases. Sinusoid spaces were narrower probably because of inflammation of hepatocyts(Fig. 2). Samples of group3(Ph) were similar to those of control group. Liver tissue was normal in this group(Fig. 3). It seems that in group4(Ns.Ph) feeding the animals with Ph(as a substance with antioxidant) has brought abnormal condition back to a normal condition(Fig. 4). As it can be observed findings of the study of enzymes activity correspond well to the results of the study of liver tissue(letters H and S in the Fig.1-4 refer to hepatocytes(liver cells) and sinusoids(spaces between hepatocytes and channels of blood which contain kupffer cells) respectively).

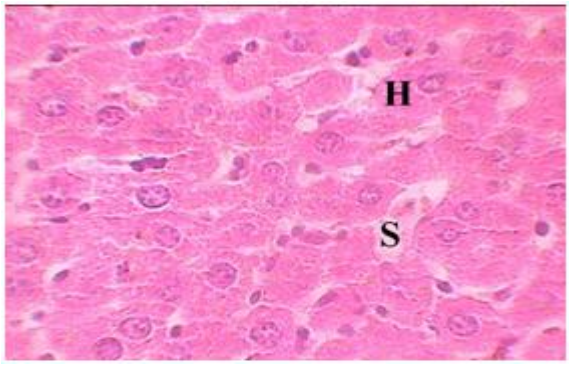

Fig. 1. Microscopic view of control group's normal liver, H. hepatocyte, S. sinosoid(stained with hematoxylin, ×1000).

Discussion

Results from histopathological and biochemical studies show damge of liver tissue in male mice treated with silver nanoparticles. Significant increase of MDA concentration in group2 (500 Ns) compared with control group(as indicated in Fig. 1) suggests that silver nanoparticles with concentration of 500 ppm can produce free radicals. Fig. 2 and 3 display CAT and GPx antioxidant enzymes respectively, which decreased significantly in group2(500 Ns), showing that nanosilver particles with concentration of 500ppm have no antioxidant property. As shown in Fig. 1, significant increase of MDA concentration in group3(Ph) compared with control group, and significant decrease of MDA concentration compared with group2(500 Ns) suggests that Ph ethanolic extract with dose of 20mg/kg/day for 30 days can produce free radicals, but less than that in group2. Activity of CAT and GPx-in Fig. 2 and 3 respectively decreased significantly in group3(Ph) compared with control group, and increased significantly compared with group2(500Ns). As displayed in Fig. 1, there is no significant difference between increased MDA concentration in group4(Ns.Ph) and MDA concentration in group3(Ph). But there is a significant difference between increased MDA concentration in group 4 and control group and also between decreased MDA concentration in group4 and MDA

concentration in group2(500 Ns). CAT and GPx activity in group4(Ns.Ph) (as shown in Fig. 2 and 3 respectively) are not significantly different from those in control group, but they show a significant increase, compared with those in groups2 and3(500 Ns, Ph respectively).The reason for malondialdehyd encrease both in group3(Ph) and in group4(Ns.Ph) is that MDA can represent the function of all oxidant and antioxidant systems among which is catalase. In group2 the formation of free radicals reduced catalase activity, and catalase activity in group4 was the same as in control group. Hence ethanolic extract 100% combined with silver nanoparticles can neutralize free radicals, while the effective compounds of this extract in solvent of 100 cannot do that alone. A possible way of increasing antioxidant property of these effective compounds is to change the type of solvent, or to modify ethanol percentage. Pathological findings of this research correspond to biochemical findings, as group3 (Ph) with antioxidant property is similar to control group. Group2(500Ns) produced free radicals, which caused peroxidation of membrane lipids-based on biochemical findings-leading to the formation of lipidic peroxides(malondialdehyde) and probably to hepatocyte cords disorder. The amount of malondialdehyde increase in mice treated with silver nanoparticles shows an increase of lipidic peroxidation that result in liver damage and also inability of defence mechanism of antioxidant to prevent excessive formation of free radicals. In the groups treated with nanosilver particles and Ph ethanolic extract, Ph (with antioxidant property) increased CAT and GPx enzymes that raised enzymic antioxidant defence mechanism against free radical resulting from silver nanoparticles. In fact, the liver tissue of this group is similar to that of control group, in other words Ph prevents liver damage.

Hansen and Nagley (2003) showed that free radicals resulting from catalytic mechanism of silver nanoparticles damage non-saturated fatty acids in membrane phospholipids through lipidic peroxidation process, and cause cell aging or apoptosis(Hansen and Nagley, 2003). In the current study increase of malondialdehyde in mice treated with nanosilver particles suggests that these particles may cause free radical formation, which leads to the production of malondialdehyde through lipidic peroxidation process.

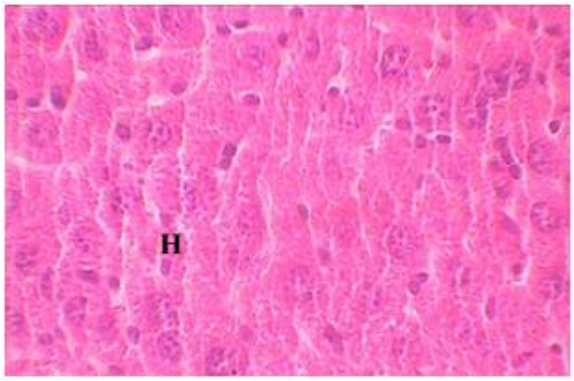

Fig. 2. Microscopic view of group2 treated with 500 NS shows that kuppfer cells in sinusoid were inflamed probably because of absorbing the nanoparticles. Sinusoid spaces are narrower and more unclear (stained with hematoxylin, ×1000).

Tang and colleagues (2008) demonstrated that injection of silver nanoparticles of various sizes results in accumulation of the particles in organs such as liver, kidney, lung, spleen, and brain. This process destroys blood– brain barrier and damages nerves (Tang and Xi, 2008). Portney and ozkan (2006) indicated that the smaller the diameter of a silver nanoparticles, the more of theme penetrates into cells and has molecular effects on inner cell mechanisms (Portney and Ozkan, 2006). Therefore, silver nanoparticels with diameter of 10 nanometer were used in the present survey for their better effects– according to portney·s studies. Muhi-eldeen and colleagues(2008) studied severe toxicity of water extract of Iraqi *Peganum harmala* L in mice. The results showed that all mice, receiving a dose of 550mg/kg through intra- mascular injection, died. There were no significance changes in red blood cells of treated and normal mice. Apparently *Peganum harmala* water extract had no toxic effect on liver and kidneys. Then, they were treated with Ph water extract through intra-mascular injection for 6 weeks. This process significant increased the number of leukocytes and neutrophils at the injection area of some Ph-treated mice (Muhi-eldeen *et al.*, 2008). Results of this study indicate that oxidative stress

caused by intra-mascular injection of Ph extract strongly stimulates cell immune system and increase the production of white blood cells in Ph-treated mice. Since the taken dosage of Ph in muhi-eldeen،s research was beyond the toxicity range of Ph water extract that is 420mg/kg, formation of free radicals increase of MDA concen tration, and decrease of antioxidant enzymes occurred due to the Ph high concentration. Accordingly, a dose of 20mg/kg/day for 30 days was determined, considering the average percentage of mice's weighs and toxicity range of Ph consequently, oxidative stress decreased and antioxidant increased. Furthermore, this amount was given orally to avoid stimulation of the immune system. In another study Zaker and colleagues (2007) showed anti-tumor and anti-reproductive effects of Ph derivatives on cancerous cells. The appropriate doses of two Ph derived compounds(harmin and harmalin) for anti-tumor activity were 0.4-1.6 and 10-6 mgr/ml respectively (Zaker *et al.*, 2007).

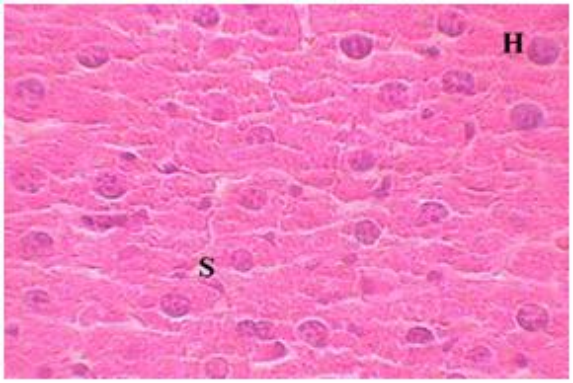

Fig. 3. Microscopic view of group3 treated with Ph ethanolic extract. It is similar to control group (stained with hematoxylin, ×1000).

In the current research, the amount of antioxidant in group 4 increases to protect against free radicals. But in group2 silver nanoparticlescause apoptosis through inducing oxidative stress, and may also lead to cancer in case of long-term contact. Accordingly, it can be suggested that polyphenol compounds of Ph can react as electron donor and show antioxidant and antiperoxidant effects through enzymic and non-enzymic mechasim. Concluding that phenolic extract prevents unnecessary reproduction of cells and also cancer by means of it،s antioxidant property. Findings of this research show that Ph ethanolic extract destroys free radicals resulting from silver nanoparticles through significant increase of antioxidant enzymes and decrease of malondialdehyde compared with group2(500 Ns). In fact, phenolic extract can be used as an antioxidant to reduce harmful effects of silver nanoparticles.

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