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Research Paper

The Effects of Oral Silver Nanoparticles and Propolis on the Level of Lipid Peroxidation in Male Wistar Rats

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ABSTRACT

Background: Oxidative stress is a leading factor in developing silver nanoparticle-induced toxicity.

Objective: This study evaluated the effects of oral prescription of different doses of silver nanoparticles and propolis on lipid peroxidation in male Wistar rats.

Methods: The male Wistar rats were randomly divided into four groups. Control group and three different treatment groups with oral prescription of 30 ppm, 60 ppm, and 60 ppm of silver nanoparticles with 200 mg/kg of propolis. The serum level of Malondialdehyde (MDA) was measured by Thiobarbituric Acid Reactive Substances (TBARS) assay using Thiobarbituric Acid (TBA).

Results: The serum level of MDA as a marker of lipid peroxidation in the control group was 1.92 mM/mL, and in other groups that received silver nanoparticles (30 ppm, 60 ppm, 60 ppm with 200 mg/kg propolis) were respectively 2.82, 3.83 and 2.62 mM/mL. MDA level also increased at the doses of 30 ppm and 60 ppm compared to the control group and decreased when propolis was prescribed with silver nanoparticles in the third group; however, its value did not reach the level of the control group. Minimal levels of serum lipid peroxidation were observed when silver and propolis nanoparticles were administered to male rats.

Conclusion: The mixture of silver nanoparticles and propolis reduces the toxic effects of silver nanoparticles; it preserves and increases the efficiency of this compound in medical applications.

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1. Introduction

Oxidative stress, caused by an imbalance between antioxidants and free radicals, has a significant impact on the occurrence and progression of various diseases by causing tissue damage. Using antioxidant compounds can significantly control this oxidative stress. In many clinical studies, new antioxidants have been discovered that encounter free radicals that aggravate some oxidant-induced diseases [1, 2].

Propolis is a mixture of different chemicals produced by honeybees, with antimicrobial, antioxidant, and anti-inflammatory properties [1, 2]. Propolis is a relatively complicated chemical substance, and its chemical components depend on the type of flora used by honeybees. Chemical analysis shows that the most important bio-active components of propolis are phenols, including flavonoids, along with other compounds such as terpenes, alcohols, and aromatic aldehydes [3]. The antioxidant properties of propolis are due to its ability to remove Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Also, the components of propolis can suppress oxidant-producing cascades by chelating metal ions present in the mentioned cascades [4]. The relationship between auto peroxidation of unsaturated fatty acids and its effect on the pathogenesis of special conditions like metabolic syndrome, diabetes mellitus, and atherosclerosis has been well-documented [5].

Silver nanoparticles, similar to other nanoparticles, have specific benefits thanks to their tiny size. The small size gives them unusual chemical and physical properties besides their particular biological activities [6]. Nanoparticles have unusual physical and chemical properties, and these such properties are only present in less amounts and not in large amounts. Silver nanoparticles are used frequently in nanomedicine, and this usage is due to their antimicrobial and anti-carcinogenic aspects. Silver nanoparticles have other biological effects, such as increasing the speed of wound healing and enhancing the immunogenicity of vaccines [7].

Silver nanoparticles have applications in cancer treatment, and they have been studied along with commonly prescribed cancer medications. Numerous studies showed the anti-carcinogenic effect of silver nanoparticles in breast, colon, ovarian, pancreatic, and cervical cancer [8]. It should be noted that silver nanoparticles impose a mild toxic danger and can cause various tissue damages. This toxic effect might affect the medical benefits of these nanoparticles [9].

Silver nanoparticles usually accumulate in the liver, spleen, and lungs. They can pass specific structures such as the blood-brain barrier and inflict cytotoxic effects. The prescription route, size of nanoparticles, method of production, and stabilizers used in producing silver nanoparticles determine the amount of toxicity [10].

One of the biological methods of producing and preparing silver nanoparticles is using plants or plant-based materials, which have a considerable amount of alkaloids, flavonoids, terpenes, and phenols. These materials with lots of antioxidants like phenols, flavonoids, and alkaloids make possible the reduction of silver salts. These organic compounds are generally used as stabilizers, and they have an essential role in the production and preparation of silver nanoparticles [11]. It should be noted that these stabilizers play a vital role in the clinical application and decline of cytotoxicity of silver nanoparticles [12].

One of the methods of producing silver nanoparticles is using propolis as a stabilizer rich in phenols, flavonoids, and alkaloids [13, 14]. Numerous studies have documented the effects of silver nanoparticles on various biochemical factors of serum or blood [15-17]. On the other hand, few studies were conducted on the effects of preparing silver nanoparticles by using propolis which is one of the main methods of preparation and production of silver nanoparticles. Since silver nanoparticles can potentially result in oxidative stress and lipid peroxidation, this can be one of the leading causes of toxicity of silver nanoparticles [18]. This study aimed to observe the effects of oral silver nanoparticles and their mixture with propolis on the serum lipid peroxidation in male Wistar rats.

2. Materials and Methods

Propolis had been frozen at first and then cut into small pieces to create propolis extract. After that, a certain amount of propolis is dried and sliced. Then, 80% alcohol was added, and the mixture was incubated for 72 hours at room temperature without direct light. The extracted liquid was filtered multiple times using a rotary device (vacuum distillation) and then incubated at 38°C, resulting in the stable propolis extract. This extract was then mixed with polyethylene glycol and fed to the rats using gavage and feeding tube in specific doses. As Figure 1 shows, the samples consist of 40 Wistar rats weighing 130 to 180 g. They were kept in clean, individual cages with free access to food and water in a standard animal laboratory. Substances were kept in a 21°C environment with a day/night cycle of 12 hours each. The rats were treated based on European laboratory animal protection guidelines [19]. Ten rats were assigned to each group. As

previously mentioned, samples were randomly selected from Wistar rats and grouped. Blood samples were also taken from the tail vein of rats, and it should be noted that the rats were anesthetized before the sampling process [20]. After sampling the blood clotted at room temperature, serum was separated by centrifugation at 5000 rpm for 5 min and stored at −80°C until MDA measurement [21]. MDA values were measured by spectrophotometric method, using Thiobarbituric Acid (TBA) and the MDA standard curve. 1,1,3,3-tetra methoxy propane was used as standard. It should also be noted that MDA reacts with TBA to produce a pink-colored solution that has a maximum absorbance at 532 nm [22].

Statistically analysis

In this study, all data are presented in Mean±SD. The software used for statistical analysis was SPSS software v. 20, and ANOVA and Turkey test were used.

3. Results

As presented in Table 1 and Figure 2, the mean and standard deviation of serum MDA in the control group and other groups (30 ppm, 60 ppm, and 60 ppm silver nanoparticle with 200 mg/kg propolis) were 1.92±0.54, 2.82±0.97, 3.83±1.1, and 2.62±1.1 mM/mL, respectively. The results indicated that by the increase of silver nanoparticles, serum levels of MDA also increase, and by adding propolis, the level of MDA is decreased.

Table 1 compares serum MDA in the control group with other treatment groups. Compared with the control group, the groups prescribed silver nanoparticles (30 ppm, 60 ppm, and 60 ppm with 200 mg/Kg propolis) had the P-values of 0.019, 0.003, and 0.097.

Variance analysis showed that the increase of silver nanoparticles without propolis results in increased levels of MDA compared to the control group while adding propolis to 60 ppm silver nanoparticles causes the decrease of MDA. Based on the level of the significant difference ($P<0.05$) and calculated P values, the increase in serum MDA is associated with the increase of the dose of silver nanoparticles, whereas adding propolis to silver nanoparticles reduces the level of MDA and shows no significant difference between the control group and 60 ppm silver nanoparticles+200 mg/kg propolis group in the level of MDA ($P=0.097$).

4. Discussion

This research studied the effect of silver nanoparticles and a mixture of propolis and silver nanoparticles on serum levels of MDA in Wistar rats. Based on the results, mean serum MDA levels in the control and inter-

Table 1. Comparison of serum MDA levels in different studied groups (mM/mL)

Groups	Mean±SD	P
30 ppm silver nanoparticles	2.82±0.97	0.019
Control	1.92±0.54	
60 ppm silver nanoparticles	3.83±1.1	0.003
Control	1.92±0.54	
60 ppm silver nanoparticles+200 mg/kg propolis	2.62±1.1	0.097
Control	1.92±0.54	
30 ppm silver nanoparticles	2.82±0.97	0.004
60 ppm silver nanoparticles	3.83±1.1	
30 ppm silver nanoparticles	2.82±0.97	0.575
60 ppm silver nanoparticles+200 mg/kg propolis	2.62±1.1	
60 ppm silver nanoparticles	3.83±1.1	0.003
60 ppm silver nanoparticles+200 mg/kg propolis	2.62±1.1	0.003

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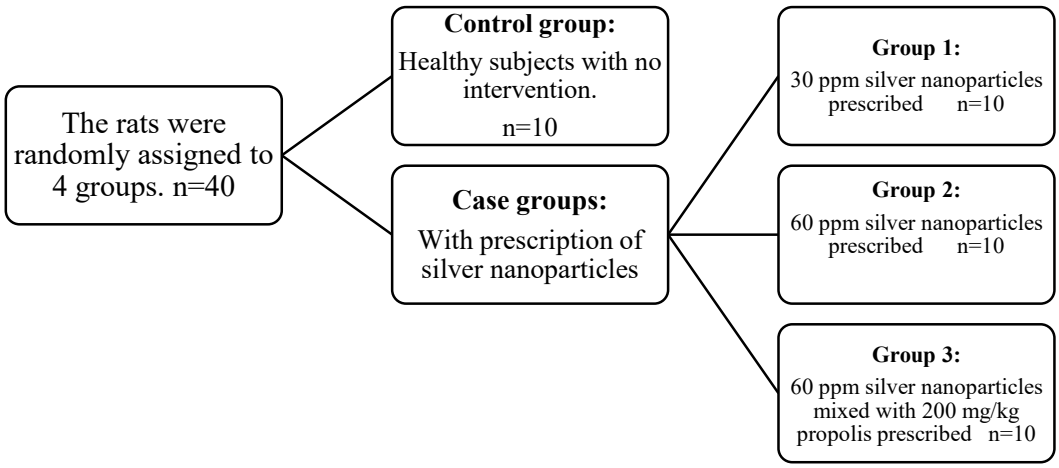

Figure 1. The diagram of the study and different groups of the study

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vention groups that received only silver nanoparticles were increased, while adding 200 mg/kg propolis to 60 ppm silver nanoparticles showed the opposite effect, and the level of MDA decreased. It can be stated that the increase of silver nanoparticles has a significant effect on serum MDA levels in Wistar rats. Our findings confirm the findings of Fuliang et al. [18] and Sameni et al. [23]. This study also showed that propolis extract has a controlling effect on serum MDA in animal models, and a dosage of 200 mg/kg of propolis had an efficient function. Chen et al. [24] also showed up-regulation of heme oxygenase, glutathione peroxidase, and superoxide dismutase genes in response to different doses of silver in a dose-dependent manner. Also, they showed a high level of apoptosis in the liver, kidneys, and spleen. In another study performed by Dănilă et al. [25], silver nanoparticles increased malondialdehyde levels and antioxidant enzymes in female Wistar rats.

Studies proved that silver nanoparticles have cytotoxic properties, increasing lipid peroxidation and malondialdehyde levels in tissues. Also, it causes additional oxidative stress and various tissue damages in different organs [24, 25]. Silver nanoparticles can have antimicrobial and anti-carcinogenic effects, documented in many studies. These toxic properties are like a double-edged sword that can overshadow the benefits of silver nanoparticles. However, propolis, which contains phenols, alkaloids, and flavonoids, and its antioxidant properties were also studied extensively [26, 27].

5. Conclusion

Based on the results of this study and the fact that propolis is a substance that can stabilize and reduce silver nanoparticles, it can be concluded that the mixture of silver nanoparticles with propolis can decrease the toxic and cytotoxic effects of silver nanoparticles. This method of preparation of silver nanoparticles can preserve and increase the efficacy of using silver nanoparticles in clinical practice.

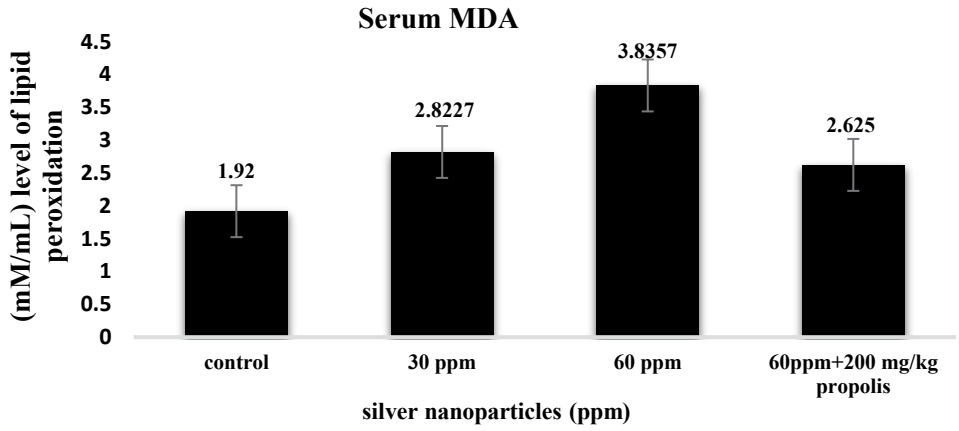

Figure 2. The effect of different doses of silver nanoparticles on the serum level of MDA

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Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of the Qazvin University of Medical Science approved the present research (No.: IR.QUMS.REC.1398.144).

Conflict of interest

The authors declared no conflict interests.

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Authors' contributions

Conceptualization and Supervision: Hossein Piri and Nematollah Gheibi; Investigation and data collection: Hossein Piri, Nematollah Gheibi, Zahra Mohammadi, and Seyed Amir Hadi Hosseini; Data analysis and interpretation: Hossein Piri and Nematollah Gheibi; Writing – original draft, and Writing – review & editing: Hossein Piri, Nematollah Gheibi, and Seyed Amir Hadi Hosseini, Ehsan Aali, Yazdan Naderi, and Seyed Amir Hadi Hosseini; Approval of the final manuscript: All authors.

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