Original Article

Evaluation of Protective and Immunomodulatory Effects of Hydroalcoholic Extract of *Scrophularia striata* on Silver Nanoparticle-Induced Toxicity in Male Rats

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Abstract

Introduction: Recently, silver nanoparticles (Ag-NPs) have found extensive and raising biomedical applications. Ag-NPs may lead to increased rate of toxicity on human health and environment. Because of the high antioxidant potential of the *Scrophularia striata*, the aim of the present study was to investigate the protective influence of *Scrophularia striata* against Ag-NPs-induced toxicity.

Materials and Methods: Thirty male Wistar rats were randomly divided into 5 groups (n=6 for each group). Group 1 was normal control rats. Group 2 received only Ag-NPs (200 ppm). In groups 3 to 5, the rats were pretreated with different concentrations (20, 60 and 180 mg/kg) of the *Scrophularia striata* extract, respectively and then were treated with Ag-NPs to induce toxicity. Animals were treated once daily by gavage over a period of 30 days. At the end of the treatment period, blood samples were collected and serum IgG, IgM, C3, C4, and CRP levels were determined. Data were statistically analyzed through one-way ANOVA, followed by Tukey's post hoc test.

Results: Oral administration of Ag-NPs evoked a significant increase in the serum IgG, IgM, C3, C4, and CRP levels, compared with those in the control group ($P<0.05$). These changes were ameliorated through treatment with *Scrophularia striata* extract at different doses as compared with the Ag-NPs-treated group ($P<0.05$).

Conclusion: The extract was found to be as an effective immunomodulatory agent against Ag-NPs-induced toxicity presumably due to its active compounds with medicinal value.

Keywords: Silver Nanoparticle, *Scrophularia striata*, Toxicity, Rat

1. Introduction

Nanotechnology research and development is a rapidly emerging field and the production of novel man-made nanoparticles is increasing worldwide [1, 2]. Nanoparticles (NPs) are defined as unique particles with at least one dimension less than 100 nm in diameter [3]. NPs can certainly enter the body via various routes: dermal penetration, ingestion, inhalation and systemic entrance. The deposition of nanoparticles in vital orga ns or tissues may cause cell damage [4, 5]. Because of the extreme small size and catalytic properties of the nanoparticles, they have great potential to interact with biological organisms by producing free radicals that induce direct and indirect effects of oxidative stress, inducing toxicity, altering the expression of genes and cellular

apoptosis [6]. Also, it was demonstrated that NPs may alter the cellular responses by passing through the cellular membranes and interaction with biomolecules. Then finally NPs lead to damage of proteins and nucleic acids [7].

Silver nanoparticles (Ag-NPs) are among the most frequently used nanoparticles in different industries such as medicinal devices, healthcare products, pharmacology, biotechnology, engineering, electronics, and energy [8]. Despite the fact that Ag-NPs possess nemerous advantages, their possible toxicity has recently become an important issue. In this regard, there are many in vitro and in vivo reports that have demonstrated toxicological effects of Ag-NPs on various tissues such as brain, liver, lung, vascular system and reproductive cells [9].

Furthermore, there is also evidence that NPs can interact with different cells of the immune system. The principal function of the immune system as primary defense barrier is detection of foreign substances, which plays significant role in the protection of host [10]. NPs can interfere with this function or can themselves be recognised as foreign antigens and therefore evoke protective immune responses [11]. Interaction between NPs and different elements of the immune system provoke cell signaling pathways, which may cause immune factor induction and immunomodulation [12]. The special properties of NPs lead to different immunotoxic effects, including membrane disruption, and release of pro-inflammatory cytokines through the production of reactive oxygen species (ROS) and oxidative stress induction [13]. The immunomodulatory effects induced by Ag-NPs has been confirmed by several in vivo studies. For example, repeated oral administration of Ag-NPs to mice increased cytokines including IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-β in serum. In addition, increase of B cell distribution in lymphocyte and IgE production were also observed [14]. A dose dependent increase in IgM and IgE serum levels was seen after 28 days intravenous administration with Ag-NPs in rats [15]. Another study showed immunotoxicity of Ag-NPs in rats, with an increase in IgG and IgM levels [16].

Medicinal plants and natural products have been used for centuries as traditional treatments for numerous diseases. Most pharmacological activities of medicinal plants are primarily attributed to their phytochemical constituents [17]. In recent years, phytochemicals with antioxidant effects have been demonstrated to ameliorate the damage of oxidative stress-associated conditions through inhibition of ROS generation and improvement of antioxidant defense mechanisms [18]. *Scrophularia striata* as an Iranian flowering plant species belongs to the Srophulariaceae family. It is cultivated in many countries, particularly in the western and northeastern states of Iran, and are used as a traditional herb for various purposes [19]. Phytochemical analysis of *Scrophularia striata* has revealed its various biologically active compounds such as phenolic acids, flavonoids (quercetin and Isorhamnetin-3-O-rutinoside), iridoids and phenyl propanoid glycoside with antioxidant activity [20]. Pharmacological activities including antioxidant and neuroprotective [19], immunomodulatory [21], anti-inflammatory [22], antimicrobial [23], anticancer [24], antinociceptive [25], antidepressant and anxiolytic [26] have been reported previously.

Based on the literature and considering the unique features of *Scrophularia striata*, the present study was conducted to determine the protective and immunomodulatory effects of *Scrophularia striata* extract in Ag-NPs induced toxicity on the rat model.

2. Materials and Methods

2.1. Characterization of Ag-NPs

The Ag-NPs used in this study were a type of nano-powder (purity>99%) and supplied from Iranian Nanomaterials Pioneers Company (Mashhad, Iran). The

sizes of Ag-NPs were determined using scanning electron microscope (SEM), indicating that Ag-NPs diameter were 20-40 nm. Ag-NPs were suspended in 0.9% saline and then sonicated using a sonicator for 30 min just before administration [1]. The Ag-NPs solution was freshly prepared every day just before use. The SEM image of the Ag-NPs is also depicted in Fig. 1.

2.2. Preparation of the Extract

Fresh leaves of *Scrophularia striata* were collected from Kermanshah Province from June to August 2019. It was authenticated by a botanist in the research herbarium of Jahad-Keshavarzi Organization, Kermanshah Province of Iran. The cleaned *S. striata* leaves were minced and air-dried at room temperature under the shade and then ground with a laboratory grinder to obtain its powder form. To prepare hydroalcoholic extract, the powder was extracted three times with the mixture of ethanol/water (70/30, V/V) for 72 h. The extract was filtered using filter paper. The solvent was evaporated and concentrated using a rotary evaporator under vacuum at 40°C. The dry extract was stored at 4°C until further use. In this study, normal saline was added to the dried extract to prepare the respective doses of the extract [17].

2.3. Experimental Animals

Thirty adult Wistar rats were purchased according to inclusion and exclusion criteria from the Animal Care Unit of Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. It is of significant importance to determine these prior to starting the study. The inclusion criteria were as follows: Male Wistar rats of 8-10 weeks of age, healthy, with normal behaviour and activity, and weighing 200 to 210 g. The exclusion criteria were as follows: female Wistar rats, unhealthy, weak male Wistar rats, and the rats that were previously used for any other experimental procedure. They were maintained in stainless steel cages (six rats/cage) under controlled environmental conditions with temperature (22±2°C), relative humidity of 55±5% and lighting (12-h light/12-h dark cycle). All rats were fed with a standard laboratory pelleted chow diet and fresh water ad libitum.

2.4. Experimental Design

The current study is a basic experimental research. This study was conducted in the biomedical laboratory, department of physiology and pharmacology at Faculty of Veterinary Medicine, Razi University from June 2019 to August 2019. The rats were acclimatized for 1 week before being used in the experiment. Then, the rats were randomly assigned into five groups with six animals per group. In group 1, animals were orally and daily administered with normal saline and served as the normal control. In group 2, animals were orally and daily administered with Ag-NPs (200 ppm) only. In groups 3, 4 and 5, rats daily received the hydroalcoholic extract of *S. striata* at doses of 20, 60 and 180 mg/kg body weight by oral gavage, respectively plus Ag-NPs. In this study, doses of Ag-NPs and *S. striata* extracts were determined according to previous reports and our unpublished pilot studies [27]. The experimental period was 30 days. All experiments used herein were approved by the Animal Ethics Committee of Razi University and followed the National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (Animal Ethical Approval Number: 396-2-038).

2.5. Sample Collection

At the end of the study period, all rats were anesthetized using intraperitoneal injection of ketamine (60 mg/kg) and xylazine (20 mg/kg). Then, their blood samples were taken directly via the cardiac puncture method. Serum specimens were separated following blood centrifugation at 2000 rpm for 10 min at room temperature and were stored at -20°C until immune parameters analysis. Serum IgG, IgM, C3,

C4, and CRP concentrations were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits in the Central Laboratory, Faculty of Veterinary Medicine, Razi University. Protocols were performed according to the manufacturer's instructions.

2.6. Statistical Analysis

The obtained data were analyzed through One-way Analysis of Variance (ANOVA) and Tukey's HSD post-hoc test using SPSS software for Windows (Version 21, Chicago, IL, USA). The results were expressed as Mean±SEM. $P<0.05$ was considered as the significant difference between the groups.

3. Results

Our results revealed that the oral administration of Ag-NPs to rats caused a significant increase in IgG and IgM serum levels compared with those in the control group ($P<0.05$). Furthermore, treatment with *S. striata* extract significantly decreased the elevated levels of parameters mentioned above in a dose-dependent manner compared with the Ag-NPs-treated rats ($P<0.05$) (Fig. 2 and Fig. 3). Also, serum C3 and C4 concentrations were significantly higher in the group receiving oral Ag-NPs as compared with the healthy control group ($P<0.05$). Administration of *S. striata* extract decreased these parameters toward the control value ($P<0.05$) (Fig. 4 and Fig. 5). Additionally, increased levels of serum CRP concentration was observed in the Ag-NPs-treated rats as compared to the control ($P<0.05$) while the *S. striata* extract treatment significantly ($P<0.05$) decreased the level of CRP compared with the Ag-NPs-treated group as shown in Fig. 6.

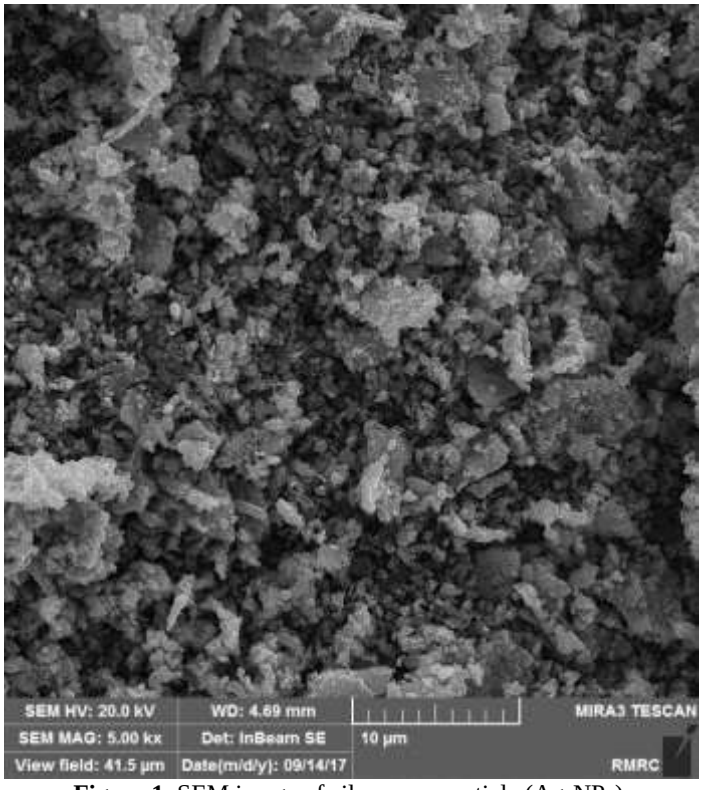

Figure 1. SEM image of silver nanoparticle (Ag-NPs).

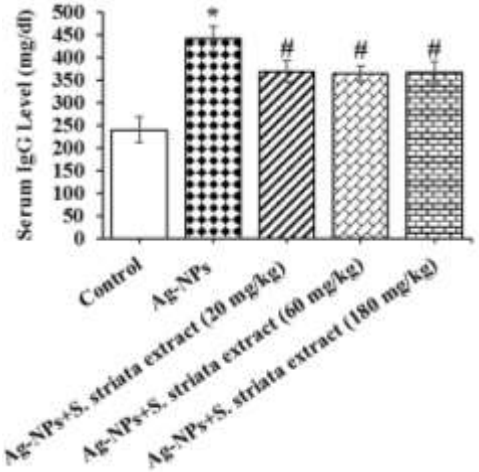

Figure 2. Effect of *Scrophularia striata* extract on serum IgG level in Ag-NPs induced toxicity in male rats. Values are expressed as Mean±SEM of six rats per group. * P<0.05: Compared with control group, # P<0.05: Compared with Ag-NPs-treated group

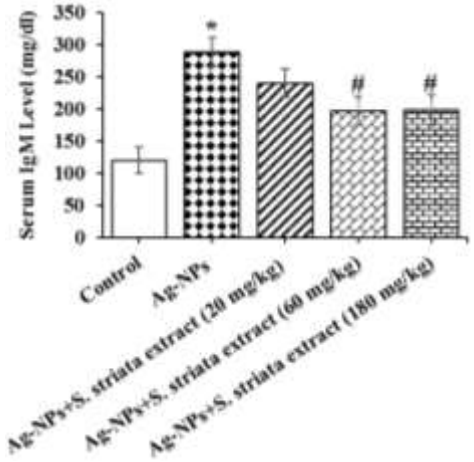

Figure 3. Effect of *Scrophularia striata* extract on serum IgM level in Ag-NPs induced toxicity in male rats. Values are expressed as Mean±SEM of six rats per group. * P<0.05: Compared with control group, # P<0.05: Compared with Ag-NPs-treated group

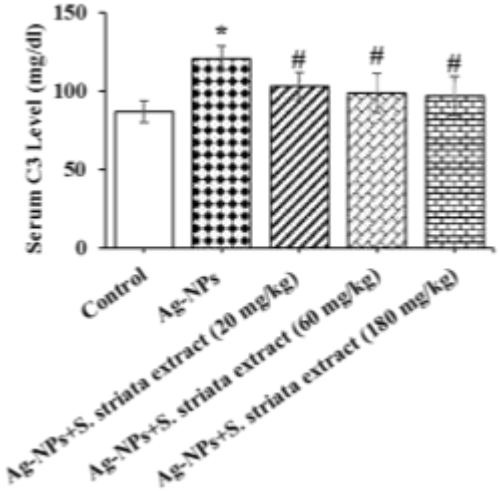

Figure 4. Effect of *Scrophularia striata* extract on serum C3 level in Ag-NPs induced toxicity in male rats. Values are expressed as Mean±SEM of six rats per group. * P<0.05: Compared with control group, # P<0.05: Compared with Ag-NPs-treated group

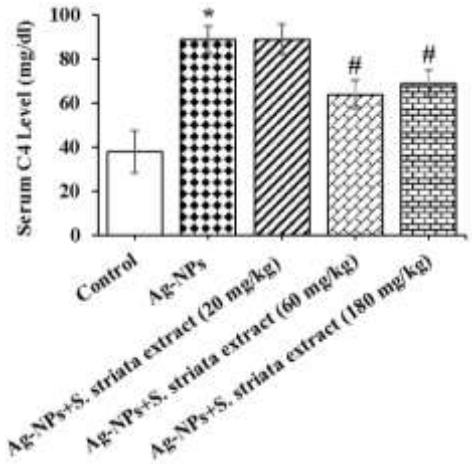

Figure 5. Effect of *Scrophularia striata* extract on serum C4 level in Ag-NPs induced toxicity in male rats. Values are expressed as Mean±SEM of six rats per group. * P<0.05: Compared with control group, # P<0.05: Compared with Ag-NPs-treated group

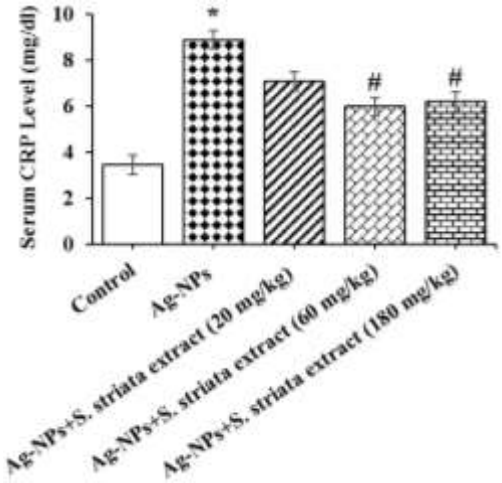

Figure 6. Effect of *Scrophularia striata* extract on serum CRP level in Ag-NPs induced toxicity in male rats. Values are expressed as Mean±SEM of six rats per group. * P<0.05: Compared with control group, # P<0.05: Compared with Ag-NPs-treated group

4. Discussion

The present study was designed for the first time to investigate the possible modulatory role of *Scrophularia striata* extract against Ag-NPs induced toxicity. According to the results obtained from experiments, the immunological parameters including IgG, IgM, C3, C4, and CRP in the plasma of rats exposed to Ag-NPs for 30 days were significantly higher than that of the control. Our study revealed that *S. striata* extract possessed protective efficacy against Ag-NPs intoxication and alleviated the changes caused by Ag-NPs. Nanotechnology research is a new and rapidly developing area of science. Current developments and various biomedical applications of nanoparticles have vital impact in many aspects of human health. Therefore, it is of concern that these particles have the potential to affect both human health and environment [28]. One of the most widely used NPs in pharmaceutical, commercial and industrial products are silver nanoparticles (Ag-NPs) [8]. Despite many therapeutic benefits of these nanomaterials, toxicological hazards are usually expectable. The toxicity of Ag-NPs is considered to depend on their unique physical and chemical characteristics, including the size, shape, chemical compositions, surface area, surface charge, and stability [29]. Further to particle-related

factors, studies have shown that the administered dose, and route of administration seem to be important parameters in NPs-induced biotoxicity [30]. The mechanism involved in the toxicological pathway of high concentrations of Ag-NPs was likely through the overproduction of reactive oxygen species (ROS) and their resultant oxidative stress, leading to the damage of DNA, proteins, and lipids and finally causing cellular apoptosis [31]. Nanoparticle-induced inflammation is one of the main toxic effects of these particles [32]. Because of the importance of inflammation as a modulator of human health, the effective and safe in vivo use of Ag-NPs is linked to interaction with immune system cells [33]. The interactions between NPs and various components of the immune system may alter immune response and cause immunostimulation and/or immunosuppression [34].

In the present study, the toxicity of Ag-NPs was investigated in rats through oral route for 30 days and results revealed that serum levels of IgG, IgM, C3, C4, and CRP were elevated in Ag-NPs-treated group. This means that Ag-NPs are recognized and processed by the immune system as foreign substances' antigens. In this regard, experimental studies have demonstrated that exposure to Ag-NPs can interact with the immune system in several ways and modify the immune system functions. For example, the prolonged (28 days) intravenous injection of Ag-NPs to rats caused a severe increase in spleen size and weight. Also, both B and T cell populations displayed an increase in absolute cell number. Additionally, suppression of natural killer cell (NKC) activity, increased IL-1β, decreased IFN-γ, IL-6, IL-10 and TNF-α production, increase in IgM and IgE antibody levels in serum, and increase of neutrophilic granulocytes in the blood were observed [15]. Another study by Park et al., 2010 [14] demonstrated that nanosized Ag particles caused an enhancement in the serum levels of cytokines such as IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-β by oral administration in mice. Additionally, distribution of B cell in blood lymphocyte and IgE antibody production were enhanced. According to these results, it is proposed that Ag-NPs may cause inflammatory responses in mice. The complement system is an important component of the immune system. This system as first line of host defense against antigens plays an important role in innate immunity and by augmenting B-cell proliferation, it also affects acquired immunity [35, 36]. Activation of the complement system occurs through three main overlapping pathways: classical, alternative, and lectin pathways. This system is composed of many pro-inflammatory proteins. Among those, C3 fragment is a critical component in activating the complement cascade, which plays a vital role in opsonization and then augments recognition and clearance of pathogens by phagocytic activity of mononuclear phagocytes [37]. Besides, C4 is the key protein of the classical complement pathway which can be activated with C3 and causes inflammation and clearance of pathogens [38]. Several lines of evidence indicate that NPs are activators of the complement system. NPs-mediated activation of complement cascade via alternative pathway may be attributed to adsorption and binding of complement molecules to their surfaces [39]. As described earlier, Ag-NPs increased serum C3 and C4 concentrations. Elevated serum C3 and C4 levels in rats may indicate an increase in their production in the liver, macrophages, and monocytes. These results are essentially in agreement with previous reports showing that activation of complement system is affected by iron oxide nanoparticles [40], silver nanoparticles [41], and zinc oxide nanoparticles [42]. Furthermore, recent evidence obtained from in vitro and in vivo studies suggests that the complement

system is closely correlated with immunoglobulins. Certain immunoglobulins such as IgG and IgM, following their binding to pathogens, lead to complement system activation with subsequent induction and progression of the inflammatory response [43, 44]. In addition, an increase in serum level of C-reactive protein (CRP) in Ag-NPs-treated group as observed in the current study, is in agreement with Ansar et al., 2017 [45], who reported that intraperitoneally administration of Ag-NPs (5 mg/kg/day) resulted in a significant increase in the serum CRP level. The elevated level of CRP as an acute-phase inflammatory protein in serum, is an indication of many pathological conditions such as inflammation [46].

S. striata is a traditional herb in Iran and has long been used effectively against various inflammatory disorders in animal models [47]. In the present study we investigated the protective effect of *S. striata* extract on Ag-NPs induced toxicity. As stated in the literature review, the toxic potential of Ag-NPs to cause inflammation and generation of ROS has been proven in previous studies. Pro-inflammatory cytokines such as IL-1β and TNF-α are involved in the inflammatory processes and their systemic production leads to inflammation. Additionally, nitric oxide (NO) and COX-2 pathways are some of the major mechanisms involved in upregulation of inflammation [48].

Another main result in our experiment was that *S. striata* extract treatment attenuated the elevated serum levels of immunological parameters mentioned above in Ag-NPs induced toxicity. In recent years, modulation of immune cell response using medicinal plant have been extensively studied. Several studies have demonstrated the inhibitory effects of *S. striata* extract on chemical mediators production such as IL-1β, TNF-α, NO and PGE2 [22, 49]. The antioxidant, anti-inflammatory, and immunomodulatory properties of *S. striata* extract have also been shown [19, 21]. In addition, based on phytochemical analysis of *S. striata*, several compounds including phenolic acids, flavonoids, iridoids and phenyl propanoid glycoside with anti-inflammatory effect have been previously reported [20, 50]. Thus, it is suggested that the protective properties of *S. striata* may appear by these phytotherapeutics agents.

Although this study had several important findings, some limitations still exist in this investigation, such as incomparable data about the property of different types of the *S. striata* extract under in vitro and in vivo conditions and methodological/ technical limitations.

5. Conclusion

The present study ascertained that the extract of *S. striata* improve the toxicological effects of Ag-NPs. The observed protective and immunomodulatory activities of hydroalcoholic extract of *S. striata* on Ag-NPs induced toxicity in male rats might be partly associated with one of the effective constituents with potent antioxidant and anti-inflammatory activity. However, clarification of the underlying cellular and molecular mechanisms involved in the protective effect of *S. striata* extract needs further investigation.

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Conflict of interest

The authors declare no conflict of interest.

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