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Impact of Silver Nanoparticles on Hematological Profiles and Hepatorenal Functions in Photosensitivity *:In Vivo*

Ozdan Akram Ghareeb[1], Rizgar R. Sulaiman[2], Salam Haji Ibrahim[3]

[1]Department of Community Health Techniques, Kirkuk Technical Institute, Northern Technical University, Kirkuk, Iraq
[2]Department of Clinic and Medicine, College of Veterinary Medicine, University of Sulaimani, Kurdistan Region, Iraq.
[3]Department of Basic Sciences, College of Veterinary Medicine, University of Sulaimani, Kurdistan Region, Iraq.

Abstract

Silver nanoparticles (AgNPs) play an essential role in nanomedicine . In the current study, the impact of AgNPs was investigated in rats with photosensitivity induced by administration of *Hypericum perforatum* (HP). A total of 36 male rats were assigned randomly into 4 groups of 9 rats each. Control group (healthy), positive control group (HP induced photosensitivity), HP+AgNPs group, and AgNPs group (positive treated only with AgNPs). A wide range of blood parameters, serum biomarkers of hepatic and renal functions, anti-oxidants enzymatic activity, pro-inflammatory markers were measured. Hematological results showed that AgNPs caused a significant increase in hemoglobin (Hb), packed cell volume (PCV), and mean corpuscular volume (MCV) with reducing of white blood cells (WBCs) down to normal level following 15 days of AgNPs treatment in photosensitized rats compared to positive control group. Interestingly, AgNPs enhanced hepatorenal functions through ameliorating effect on the serum markers of hepatic and renal function at 15 days of treatment compared to positive control group. In addition, they markedly reduced the oxidative stress with obvious free radical scavenging activity at 15 days of treatment compared to positive control group. Also, results showed a significant decrease in pro inflammatory cytokines in AgNPs treated photosensitized rats, compared to positive control group. In brief, these finding would provide a new insight into the role of AgNPs that enhances the immunological, hematological, enzymatic antioxidant activity, and liver and kidney functions in photosensitized rats, which potentially could be beneficial to amend the clinical results of photosensitivity.

Keywords: *Photosensitivity, silver nanoparticles, cytokines, oxidative stress.*

Introduction

Medications induced skin sensitivity to light attracts increased attention. In past years, photosensitivity was reported through a range of drugs, and it is now recognized as a medical problem worth stopping by both researchers and drug makers. It results when certain chemicals, drugs, or herbs are systematically or topically administered at the same time when a person is exposed to ultraviolet light (UVR) or visible light, which leads to the generation of free radicals (a phototoxic reaction) or changes the drug's structure to a form that causes an immune response (photoallergic reaction) [(1-3)]. *Hypericum Perforatum* (HP) is an over-the-counter antidepressant that contains hypericin, which is known to be a highly effective photosensitizer when exposed to radiation with ultraviolet or visible light. *In vivo* studies have determined that hypericin is a phototoxic substance for the skin [(4-6)]. So, researchers have

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sought to use novel natural compounds that can provide an effective, reliable and inexpensive treatment option in the management of diseases. The application of nanotechnology in medicine is a healthcare revolution [7,8]. As it has the potential to significantly improve some of the current medical diagnosis, disease treatment and prevention. It bridges the gap between pharmacological limitations and the therapeutic potential of natural phytochemicals by improving compound targeting, pharmacokinetics, efficacy, and cellular uptake [9-11]. Among the metallic nanoparticles, silver nanoparticles (AgNPs) have antimicrobial, catalytic, and other properties, which makes it possible to apply in medicine and pharmaceutical industry [12,13]. The biological method is environmentally friendly, low-cost and does not require expensive devices because reducing and capping agents are derived from nature such as plants and microorganisms [14,15]. The use of medicinal plants is highly preferred in silver nanoparticle biosynthesis due to its versatility with strong antioxidant properties [16]. Saffron, the dark red dried stigma of *Crocus sativus* L., was chosen for the current experimental study, and it is a perennial herb without stem that belongs to the family *Iridaceae* [17]. In traditional medicine, as well as in modern pharmacology, saffron has been used in the treatment of many diseases[18]. Recently, many recent studies *in vitro* and *in vivo* have proven that saffron has many supposed biological activities, such as antioxidant, anti-inflammatory, anti-tumor, anti-allergic, anti-genotoxin, anti-bacterial, anti-diabetic, and anti-neurotoxic[19-21]. This study is considered as the first one which included the designed to evaluate the efficiency of AgNPs synthesized by Saffron on the hematological, antioxidant, immunological and hepato-renal parameters in induced photosensitivity *in vivo*.

Materials and Methods

Materials and Chemicals

Hypericum perforatum (ST. John's Wort- Herb Extract), manufactured by Solgar Inc., Leonia, NJ 07605 U.S.A. Silver nitrate, AgNO3, was obtained from Sigma Aldrich and used without further purification. All other reagents were of analytical grade with maximum purity. All glassware's were properly washed with distilled water and oven-dried before use. Saffron has been collected from the market.

Preparation of green silver nanoparticles (AgNPs)

Colloidal AgNPs solution was synthesized according to a method previously described using a green biosynthesis method for aqueous extract of *Crocus sativus* L act as a reducing and capping agents[22]. Scanning electron microscopy (SEM) confirmed that the shape of AgNPs was spherical and variable in size (15-28 nm). The concentration of silver ions determined in the colloidal solution was 21 mg/ml (examined by Atomic absorption spectroscopy flame-AASF), where this concentration was considered the stock.

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Animals and experimental design

Thirty six healthy adult male rats aged between 16-24 weeks, weighing 175-225gm., were used in this study. The animals were obtained from the animal house of Kirkuk Technical College, Northern Technical University, Iraq. They were housed in polypropylene cages under the quality laboratory condition (24±2°C, 12 hrs. light/dark cycles, with humidity 60-70%). The rats fed standard pellet feed and water ad libitum. They were acclimatized to laboratory conditions for one week before the outset of the experiment. All rats were exposed to direct sunlight daily for 30 minutes in the time between 10 am - 12 pm. In the summer duration lasts 15 days. They were randomly divided into four groups with nine animals in each group .The experimental design was as follows :

- **Control group (I)** : Healthy rats (without photosensitivity).
- **Positive Control group (II)**: Rats induced with photosensitivity by giving HP (3mg/kg B.W orally) for 15 days. In this group rats were left as untreated and not exposed to direct sunlight.
- **HP+AgNPs group (III)** : Rats were co-administered with a combination of HP (3mg/kg B.W orally) and AgNPs (100 mg /kg B.W orally) simultaneously for 15 days.
- **AgNPs group (IV):** Rats were administered with HP (3mg/kg B.W orally) to induce photo-sensitivity and then treated with AgNPs only (100 mg/kg B.W orally) for 15 days.

Hematological and Serological Analysis

Blood count was performed by automatic hematology analyzer to determine different hematological parameters such as white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HB), packed cell volume (PCV), and mean corpuscular volume (MCV). Serological parameters were standardized by diagnostic kits (Roche) in an automatic chemistry instrument. Liver function was assessed by measuring of enzymatic activity, including alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, proteins, albumin, globulin concentration. Kidneys function was assessed by measuring of blood urea, creatinine, and uric acid. Antioxidant system was assessed by measuring enzymatic activity of glutathione (GSH) and catalase (CAT), and oxidative stress parameter malondialdehyde (MDA). Cytokines including serum interleukin 1alpha (IL-1α), serum interleukin – 6 (IL -6) and tumor necrosis factor-alpha (TNF-α) were measured.

Statistical analysis

All results are presented as mean ± S.D. where *n* reflects the number of experiments. Graph Pad Prism 5 software (Graph Pad Software, USA) was used for data presentation and statistical analysis, and either two- way ANOVA or one-way ANOVA (with Tukey‟s multiple comparison *post*-test) used where applicable. P values of <0.05 and <0.0001 were considered statistically significant.

Results

Although there was no significant change in RBCs value ($P>0.05$) in all groups, there was a significant decrease ($P<0.001$) in PCV and MCV with slight decrease in Hb estimation in rats with induce photosensitivity, compared to control group. Interestingly, treatment of photosensitized rats by AgNPs

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for 15 days showed a significant increase in Hb, PCV and MCV compared to non-treated rats, almost similar to those value in control healthy rats. Hematological estimation also showed that total number of WBCs was significantly increased in photosensitized rats, compared to control group. However, treatment of photosensitized rats by AgNPs restored the total number of WBCs almost to the normal value, which was significantly decreased compared to positive control group. As shown in figure 1.

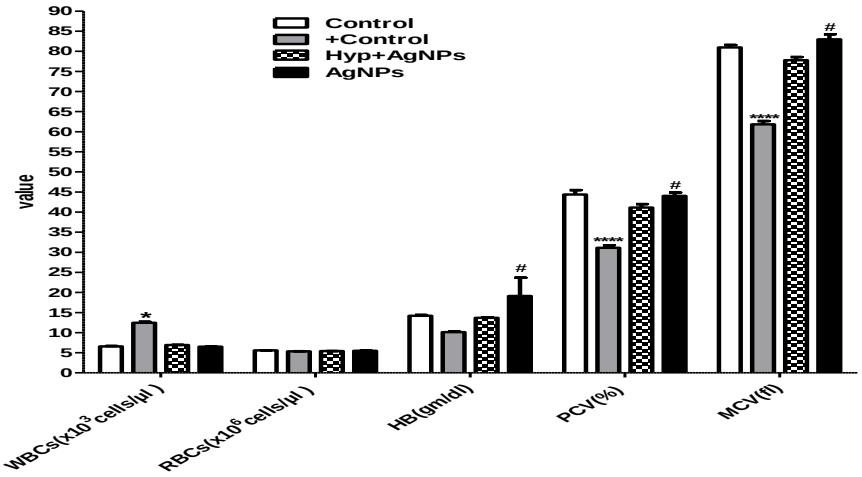

Figure 1: Effect of AgNPs on hematological profiles in photosensitized rats, both in AgNPs treated and non-treated groups. Data are shown as Mean± SEM.*P<0.001 compared to control and treated groups, #P<0.05 compared to HP+AgNPs treated group, n=9 for each group.

Results showed that photosensitivity caused a significant increase (P<0.001) in the level of serum AST, ALT , ALP , and bilirubin compared to control group. However, treatment of photosensitized rats by AgNPs showed a marked decrease (P<0.001) in the level of these parameters, almost to the same level as in control group compared to positive control rats. There was no significant difference in rats with induced photosensitivity that co-administered with HP and AgNPs simultaneously compared to AgNPs treatment on its own alone, as shown in figure 2.

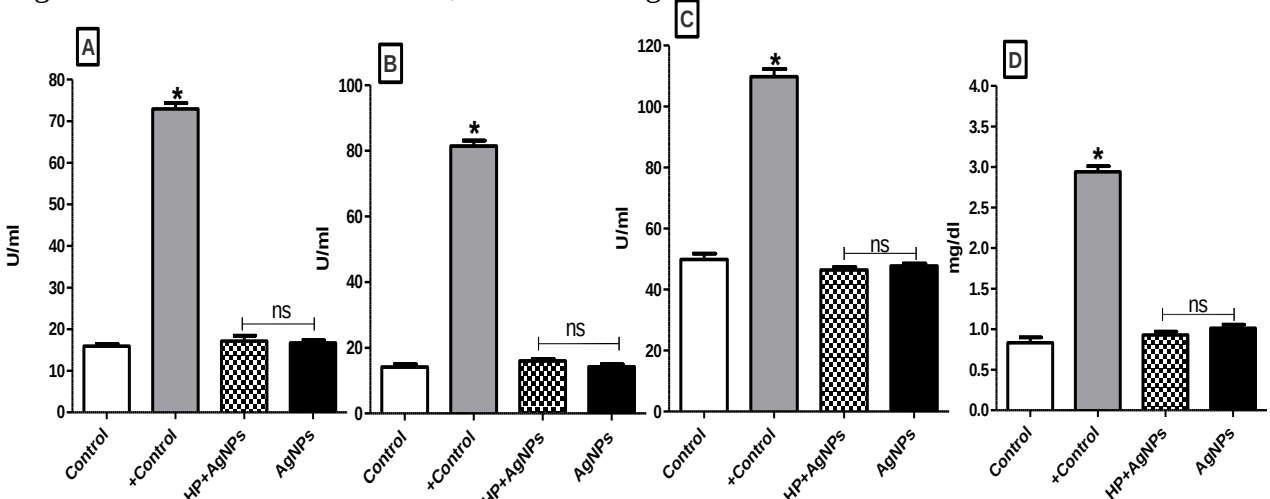

Figure 2: Effect of AgNPs on serum enzymatic markers of hepatic function including (A) AST, (B) ALT, (C) ALP, as well as (D) bilirubin levels in photosensitized rat compared to control group. Data are shown as Mean± SEM.*P<0.001 compared to control and treated groups, n=9 for each group.

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Positive control group showed a significant decrease (P<0.001) in serum level of total protein, albumin and globulin, compared to control group. Interestingly, treatment of photosensitized rats by AgNPs revealed a marked increase (P<0.001) in the level of serum protein, albumin and globulin compared to positive control group, almost to the same level as in control healthy group. There was no significant difference between AgNPs treatment groups compared to control groups. As figure 3.

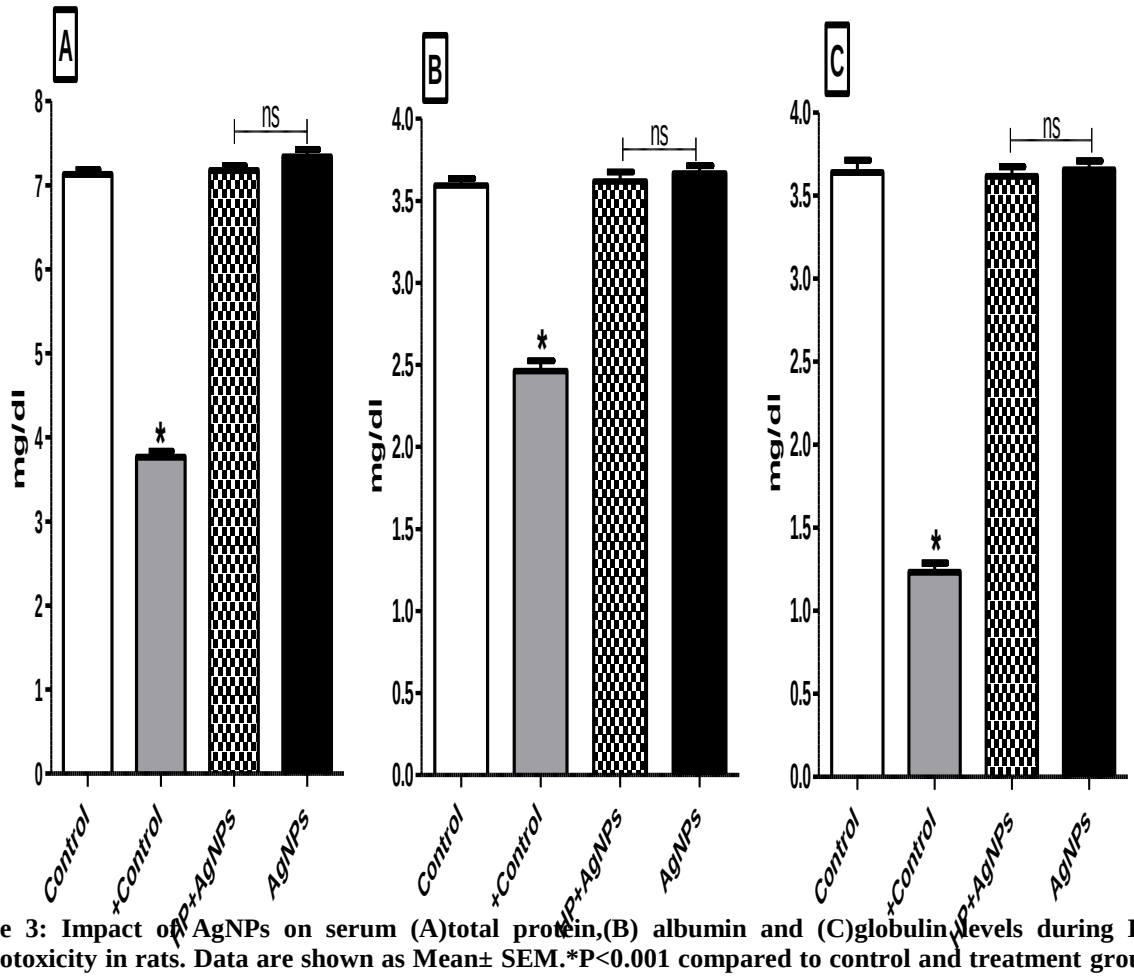

Figure 3: Impact of AgNPs on serum (A)total protein,(B) albumin and (C)globulin levels during HP induced hepatotoxicity in rats. Data are shown as Mean± SEM.*P<0.001 compared to control and treatment groups, n=9 for each group.

In positive control rats, results exhibited a significant increase (P<0.001) in the serum level of urea, creatinine and uric acid, compared to control rats. However, treatment of photosensitized rats by AgNPs did restore the kidney functions by producing marked decline in the level of serum urea, creatinine and uric acid, compared to positive control rats, almost to the same level as in control healthy rats (Figure 4). Since AgNPs obviously enhanced both liver and kidney function in photosensitized rats, it was worth investigating the influence of AgNPs on the enzymatic antioxidant activity in positive control rats.

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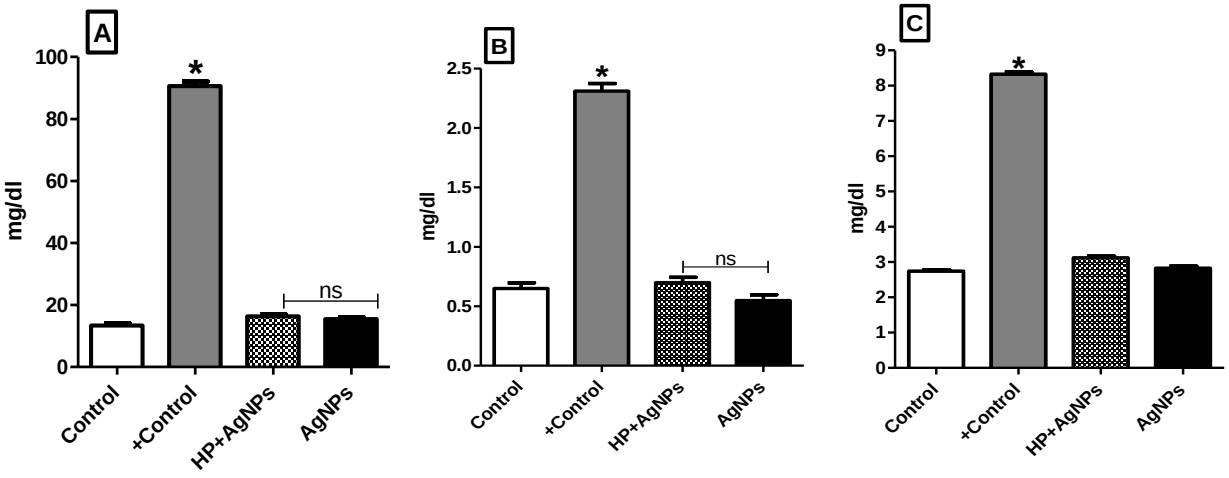

Figure 4: Recovery of kidney function in photosensitized rats following treatment by AgNPs. Serum level of (A) urea, (B) creatinine, and (C) uric acid were significantly increased in photosensitized rats, while AgNPs markedly decreased kidney function parameters to normal value. Data are shown as Mean± SEM.*P<0.001 compared to control and treatment groups, n=9 for each group.

Current results revealed a significant increase (P<0.001) in the level of oxidative stress, MDA, in photosensitized rats compared to control group. AgNPs markedly eliminated the oxidative stress by significantly reducing the MDA level in photosensitized rats compared to non-treated rats in positive control group (Figure 5-A). Interestingly, the antioxidant enzymatic activity of GSH and CAT were significantly increased in photosensitized rats in response to AgNPs treatment, compared to non-treated rats in positive control group, almost to the same level as in control healthy rats (Figure 5-B and C).

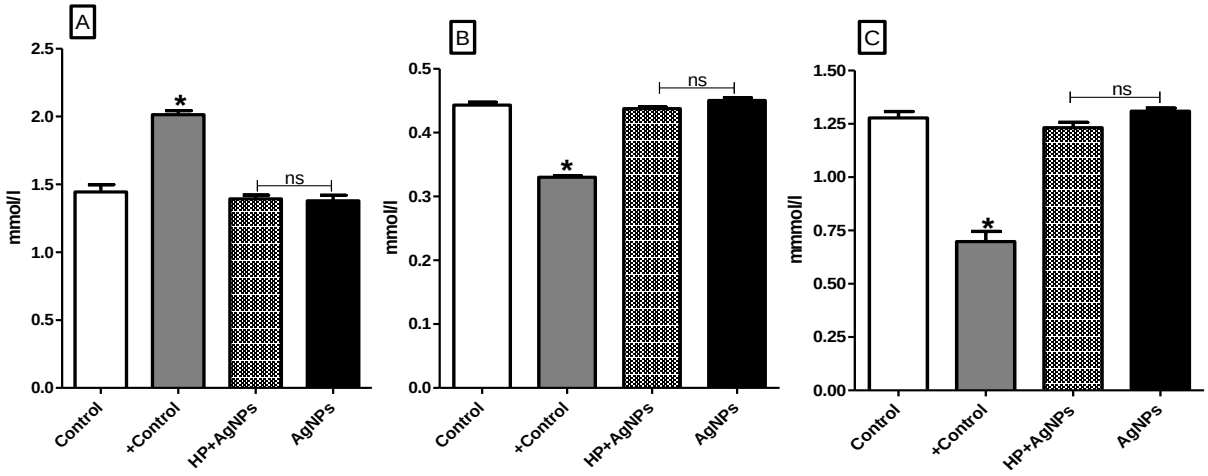

Figure 5: Effect of AgNPs on the oxidative stress and enzymatic antioxidant activity. Summary of the effect of AgNPs on (A) oxidative stress MDA, and enzymatic activity of (B) GSH and (C) CAT in rats with photosensitivity compared to control group. Data are shown as Mean ±SEM.*P<0.001 compared to control and treatment groups, n=9 for each group.

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In positive control rats, the pro-inflammatory cytokines were significantly elevated (P<0.001) compared to control healthy rats (Figure 6-A). However, AgNPs did significantly reduce the level of pro-inflammatory cytokines in photosensitized rats compared to non-treated rats in positive control group, which was almost identical to that seen in normal healthy rats in control group (Figure 6).

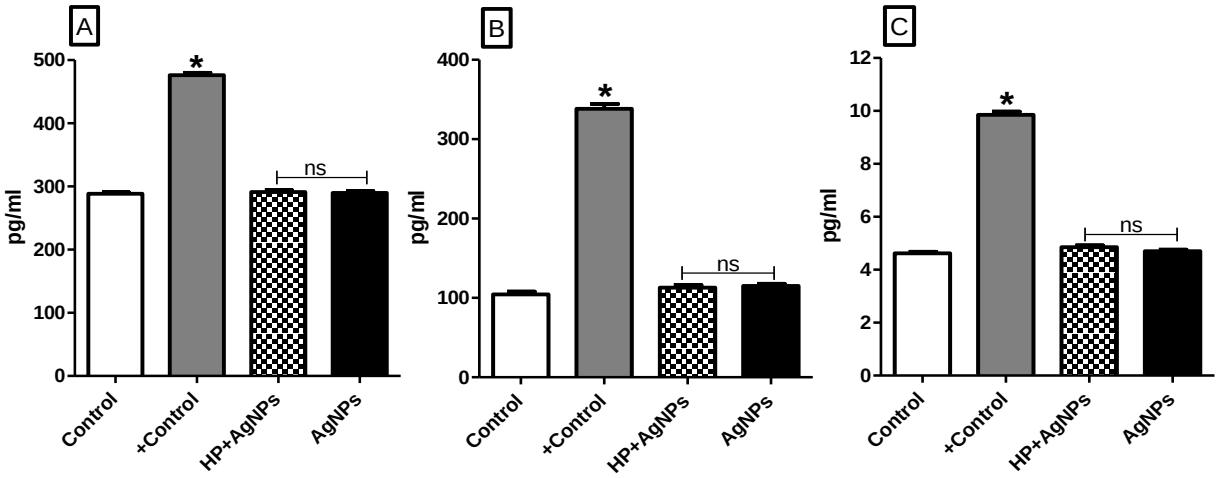

Figure 6: AgNPs reduced the serum levels of pro-inflammatory cytokines in rats with photodermatitis. Summary of the effect of the AgNPs on (A) IL1, (B) IL-6 and (C) TNF-α in rat with photodermatitis. Data are shown as Mean ±SEM.*P<0.001 compared to control and treatment groups, n=9 for each group.

Discussion

In principle, solar radiation is the most important environmental stress, as UV rays can penetrate the skin and blood[23]. Among the biological targets of photosensitivity are the cell membranes, the organelles of the cytoplasm, and the nucleus, which give rise to minor effects such as the skin. The reactions or severe effects resulting from them, such as genetic mutations, melanoma and others, may not always relate to the areas exposed to light only, but also to the internal organs[24]. The results have shown that HP induced photosensitivity caused anemia in rats since total RBCs count, Hb measurement, PCV and MCV were obviously decreed compared to control group. Here, researchers demonstrated that HP lead to hemolytic anemia via hematological toxicity, which could be attributed to the generations of free radicals that drops the integrity of the cell membrane, and bone marrow necrosis with marked decrease in the number of hematopoietic elements [25,26]. However, total WBCs count was markedly increased in rats with photodermatitis induced by HP. One possible explanation could be through immunomoduclatory effect of the HP on the lymphocytes in order to increase the WBCs and boost the immune response [27,28] . In addition, it has been found that bone marrow necrosis, produced by HP plays a vital role in the development of granulocytic leukocytosis [29]. Interestingly, application of AgNPs either co-administered with the HP or on its own alone in photosensitized rats caused a significant improvement in all hematological parameters, almost to the same level as in control healthy rats. This is consistent with the beneficial effects of AgNPs on hemato-bicochemical recovery in illness condition [30] and vital role of AgNPs in stimulating of hematopoiesis [31].This would suggest

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that AgNPs play a crucial role in the regulation of hematopoietic process, potentially through amending defect induced in the bone marrow hematopoietic stem cells.

On the other hand, photosensitivity caused by HP caused a significant increase in the serum levels of the liver functional markers including ALT, AST, ALP and bilirubin. This might be due to leakage of these cytosolic enzymes and bilirubin in to the circulatory system following hepatic cell damage since impairment of liver function and hepatocellular destruction potentially lead to alteration in the permeability of hepatocellular membrane [(32)]. However, treatment of photosensitized rats by AgNPs, both on its own alone and co-administered with HP caused a marked reduction in the serum level of hepatic enzymes and bilirubin level almost to the normal level, compared to control healthy rats. This could be potentially through stimulation of AgNPs mediated restoration of hepatic function and vital role of AgNPs in preventing hepatocellular damage via reducing the harmful effects of various toxins[(33)]. Similarly, the present study showed that total protein, albumin, and globulin levels were significantly decreased in photosensitized rats, which further reinforce the fact that photosensitivity responsible for the impairment of hepatocellular function[(34)]. Interestingly, treatment of photosensitized rats by AgNPs produced a significant elevation in serum total protein, albumin, and globulin levels to the same level as in control healthy rats, potentially through regeneration of liver function .The results also indicated that HP induced photosensitivity in rats produced a marked increase in the level of kidney function markers including blood urea, creatinine and uric acid, which are effective in proper evaluation of renal glomerular and tubular function [(35)]. These markers might be elevated during impairment of renal function [(36)] .This is consistent with previous studies by Neshat et al., who demonstrated that HP at 60mg/kg body weight enhanced the inflammation in the kidney and augmented tubular cell necrosis and glomerulosclerosis[(37)]. Treatment with AgNPs showed reduction in the serum level of renal function markers down to the normal level, indicating the effect of AgNPs on restoring kidney function and ameliorating renal damage potentially through inhibition of renal oxidative stress [(38)] and antioxidant activity of AgNPs [(39)] since production of free radicals results in oxidative induced renal injury [(40)] and might proceed to necrosis and apoptosis in renal tubular epithelial cells[(41)]. Interestingly, results of current study showed that HP markedly augmented oxidative stress through MDA elevation and reduced the activity of antioxidant system through decline in GSH and CAT levels in photosensitized rats. This further reinforces the fact that oxidative stress and free radicals were responsible for the development of renal and hepatocellular damage in photosensitized rats. However, results of current study showed that AgNPs caused a significant decrease in oxidative stress and marked elevation in antioxidant activity in photosensitized rats . Consistent with this, it has been shown that AgNPs play a vital role in reducing the oxidative stress via free radical scavenging activity that are produced in the[(42)], thus prevents accumulation of free radicals and maintains normal cellular and tissue activity [(43)]. Furthermore, it has been demonstrated that the main source of oxidative stress during kidney damage is derived from invading of immune cells as infiltrating leukocytes invade the renal glomerulus and perivascular fragments, which is followed by releasing of a wide range of proinflammatory cytokines[(44)]. This provide strong support that an increase in oxidative stress in rats with HP induced photodermatitis is clearly responsible for marked elevation of serum proinflammatory cytokines including IL1, IL-6 and TNF-α compared to control rats. Treatment of rat with AgNPs

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significantly reduced the proinflammatory cytokines down to the normal level, which could potentially be through anti-inflammatory and antioxidant properties of AgNPs since over accumulation of free radicals initiates an inflammatory response and programmed cell death[45]. In addition, it has been shown that AgNPs play a crucial role in reducing the inflammatory markers and suppressing the inflammatory events [46] as well as reduction in the level of cytokines[47].

Conclusions

The present work provides further evidence and insights into the novel role of AgNPs as antioxidant and anti-inflammatory substance in rats with HP induced photosensitivity. In addition, AgNPs play a crucial role in improving the hematopoiesis process and hepatorenal function following the HP induced photosensitivity in rats. The current results re-enforced the novel use of the AgNPs as a biological enhancer for a wide range of body functions photosensitized rats.

Inevitably, there are a wide range of future experiments that need be performed in order to strengthen the conclusions made from the current study and to provide further information into the effect of AgNPs on cellular and molecular regulatory mechanisms of hematopoiesis, as well as histopathological investigations of liver and kidney in rats with HP induced photosensitivity.

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