STUDY OF IMMUNOSTIMULATORY POTENTIAL OF SILVER NANOPARTICLES

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Abstract –*Silver nanoparticles are among the most studied nanoparticles in biological research due to their promising applications in medicine, biosensing, cancer therapy, treatment of bacterial diseases, DNA sequencing and many more. In this study we investigated the effect of silver nanoparticles in the blood stream of a mammalian system upon direct injection of chemically synthesized silver nanoparticles. Firstly, silver nanoparticles were synthesized chemically through the reduction of silver salt in the laboratory. Then, 12 rabbits were injected with silver nanoparticles and subjected to blood profiling tests. The test result showed a significant increase in the total white blood cell (WBC) count in all the rabbits injected with silver nanoparticles. The result showed that silver nanoparticles are immunostimulatory for the mammals as they can stimulate the formation of white blood cells. This immunostimulatory behavior of silver nanoparticles can be an indication of toxicity of silver nanoparticles to the mammalian systems. However, this very property of the silver nanoparticles can also be potentially utilized in cancer therapeutics.*

Key Words: Silver nanoparticles (Ag-NPs), immune stimulation, toxicity, mammalian immune response, WBC

1.INTRODUCTION

Nanoparticles exhibit significantly different properties than their bulk counterparts.[1]Nanotechnology has been introduced as a field of research due to this difference. Owing to the easily tunable morphologies of nanoparticles, they have shown huge potential in biological research.

Previous research has established that Ag-NPs potentially have a large range of applications extending from catalysis [2], plasmonics[3], optoelectronics[4], Biosensor systems [5], antimicrobial activities [6], sequencing of DNA [5], Surface-Enhanced Raman Scattering [5],clean water technology [7], energy generation [8], information storage [9] to various other biomedical applications [10].

Nanoparticles, including silver nanoparticles are known to have immunomodulatory effects inside living hosts. Once inside a living system, silver nanoparticles interact with different immune cells and can lead to potential activation of immune responses within the hosts system. Further, nanoparticles can also interfere with the ability of immune system to recognize other immunogenic substance and thus behave as immunomodulatory agents that can lead to either suppression or activation of immune responses.[11]

In this research we studied the effect of silver nanoparticles upon its injection to the blood stream of a mammalian system. The research aimed to evaluate the effect of silver nanoparticles in the immune response of a mammalian system to elucidate the impact of use of silver nanoparticles in the mammalian system as well as to gain an insight into other potential applications of silver nanoparticles.

2.EXPERIMENTAL PROCEDURE

2.1 Test Organisms

A total of 15 male rabbits weighing between 1.8-2.4 kgs of 6-8 months old each were used in the experiment. They were obtained from Department of Plant Resources, Kathmandu. The experiment was conducted in the Department of plant resources.
All the standard rules and regulations were followed in the experiment. Due consideration was taken for the use of mammals in the experiment as described by Reilly *et al.* [12]and Kim *et al.*[13]

2.2 Chemicals and Media

All the chemicals and materials used in the experiment was purchased from Eureka International Pvt Ltd.

2.3 Synthesis of Silver nanoparticles (AgNps)

Silver nanoparticles were synthesized by a method that was used in our earlier research[14]. The method used a modifications in the methodology adopted by Pal, Tak and Song, 2015[15] and involved the reduction of silver nitrate.
In the method, a seed solution was initially prepared by dissolving 0.5 ml of 10 mM $NABH_4$ in o.5 ml of $AgNO_3$ (0.01M) and 20 ml of 0.001M sodium citrate. After stirring 5ml of 0.01 M AgNO3 was then added to a mixture

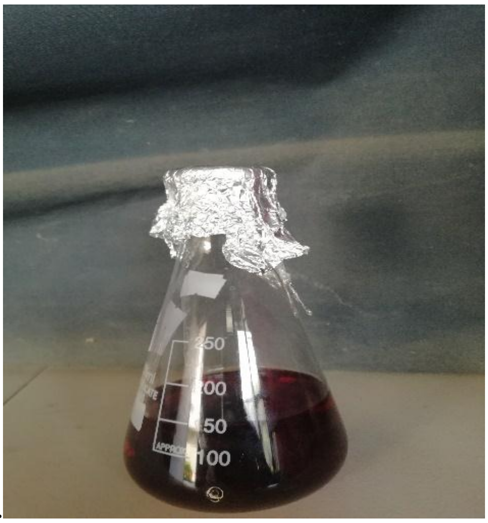

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Fig -1: aged solution of silver nanoparticles the reaction mixture continuously for 5 minutes, it was settled to age for 1.5 hours. [15].

of 10ml of 0.1M of ascorbic acid, 146ml of 0.1M CTAB and 5ml of silver seed solution to make the particle growth solution. Finally, 1ml of 1M sodium hydroxide solution was added to the particle growth solution thus formed. Within few minutes, colour change as observed from light yellow to brown, red and green within few minutes. The final mixture was successively left to age at 21°C for 12 hours, 35°C for 5 minutes and 21°C for 24 hours. The aged solution changed its colour from green to red. Subsequent centrifugation at different speeds was performed to purify the solution thus formed. Firstly, the surfactants and the small particles were subjected to centrifugation at 2100xg for 10 minutes. After suspending the resultant precipitate in water, it was again centrifuged at 755xg for 10 minutes. The precipitate, which consisted of silver nanoparticles was then suspended in water for further usage in the experiment.

2.4 Material Characterization

For the material characterization, the nanoparticle solution was placed in a 1 cm cuvette and UV/Visible spectroscopy was performed by noting the absorbance of nanoparticles at different wavelengths to find out the characteristic λ_{max} of thus synthesized nanoparticles. The spectroscopy was conducted in the chemistry laboratory of ASCOL (Amrit Science College) at Lainchaur, Kathmandu

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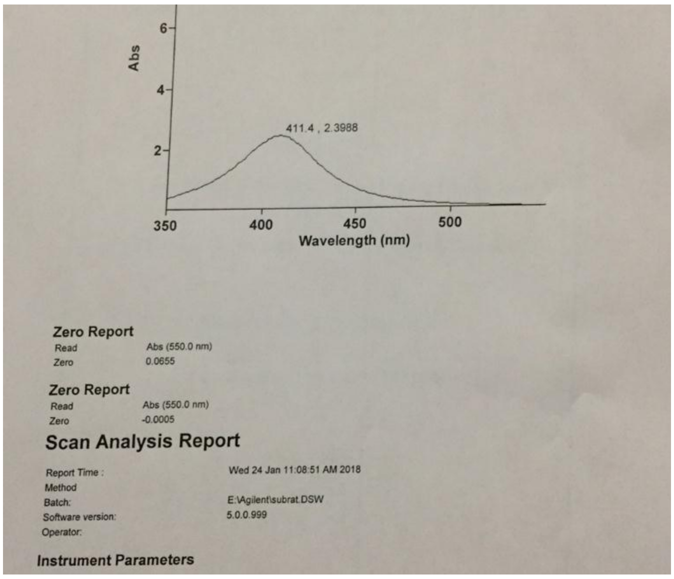

Fig -2: The absorbance spectrum of nanoparticles

2.5 Nanoparticles inoculation and blood profiling

Firstly, the blood from marginal vein of ear were collected from each rabbit and submitted to Central

Veterinary Hospital, Tripureshwor, Kathmandu for the blood profiling. Then 10ml of Ag-NPs (1mg/ml) was orally administered to twelve of the rabbits every 24 hours for 4 days. For control normal saline were injected into the blood stream of 3 rabbits. The blood from each rabbit was collected on the fifth day and submitted to the laboratory for blood profiling.

3. Results and Discussions

3.1 Physical characteristics of nanoparticles

Figure 1 shows the silver nanoparticle thus synthesized which was deep red in color.

3.2UV/Visible Spectrophotometry

Figure 2 shows the scan analysis report of the spectroscopy performed on the synthesized nanoparticle. The peak value of the absorbance for the silver nanoparticles was observed at 411 nm which was the (λ_{max}).
As explained in the previous research by the authors [14], the observed peak of absorbance λ_{max} of the synthesized solution was characteristic to silver nanoparticles and was comparable to the reference journal by Pal, Tak and Song, 2015[15]. This was used as a confirmation that silver nanoparticles had been synthesized in the process.

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Moreover, based on plasmon resonance shifts, the size of the synthesized particles was estimated to be around 14nm.

3.3 Blood Profiling reports

Reports from the blood profiling before and after the nanoparticles' injection showed a significant difference. Control rabbits (injected with normal saline) showed no significant difference in the blood profiling report.
The total WBC counts for those injected with silver nanoparticles are presented in the table 1.

Table 1: WBC counts of rabbits (1-12) before and after injection of silver nanoparticles

	Total WBC (*10^9/L) *Normal range: 3.0-11.5*		**Lymphocyte(*10^9/L)** *Normal range: 2.0-9.1*		**Monocytes(*10^9/L)** *Normal range: 0.0-0.5*		**Neutrophil(*10^9/L)** *Normal range: 0.0-2.8*	
	Bef.	Aft.	Bef.	Aft.	Bef.	Aft.	Bef.	Aft.
1	9.40	12.56	6.42	9.35	0.18	0.52	2.82	2.85
2	5.43	12.83	3.30	9.98	0.17	0.70	2.06	2015
3	8.18	12.13	5.39	9.24	0.15	0.61	2.14	2.81
4	5.21	11.97	3.28	9.27	0.14	0.60	2.02	2.89
5	10.51	12.96	7.62	9.49	0.20	0.62	2.74	2.91
6	7.94	12.66	5.13	9.18	0.18	0.59	2.61	2.84
7	9.96	12.86	7.22	9.51	0.12	0.51	2.62	2.90
8	8.86	12.78	6.10	9.43	0.22	0.59	2.54	2.81
9	7.61	12.51	5.24	9.25	0.10	0.50	2.32	2.81
10	8.99	12.97	6.69	9.58	0.23	0.61	2.12	2.94
11	11.31	13.98	8.42	10.3	0.29	0.72	2.62	2.98
12	8.79	12.69	6.13	9.27	0.01	0.40	0.05	2.85

3.4 Discussions

Effects of silver nanoparticles in immune system were particularly evident through blood profiling of rabbit models before and after silver nanoparticles treatment. It has been established that nanoparticles are able to penetrate the tissues and enter the lymphatic system owing to their size. Previous papers describe the immunostimulatory effects of silver nanoparticles to be a result of activation of complement system, cytokine release, allergenicity and so on.[16]. Toll-like receptors (TLR) and their ligands are one of the main players in the initiation of innate immunity which precedes, and is required, for the establishment of adaptive immunity. It has recently been studied whether AgNPs with a narrow size distribution had any functional impact on specific TLR stimulation of Interleukin-6 (IL-6) secretion. It was also found that AgNPs are responsible for production of various cytokines from T cells. [17] Our synthesized NPs has narrow size which implies that it could be responsible for TLR stimulation and has role in stimulating adaptive

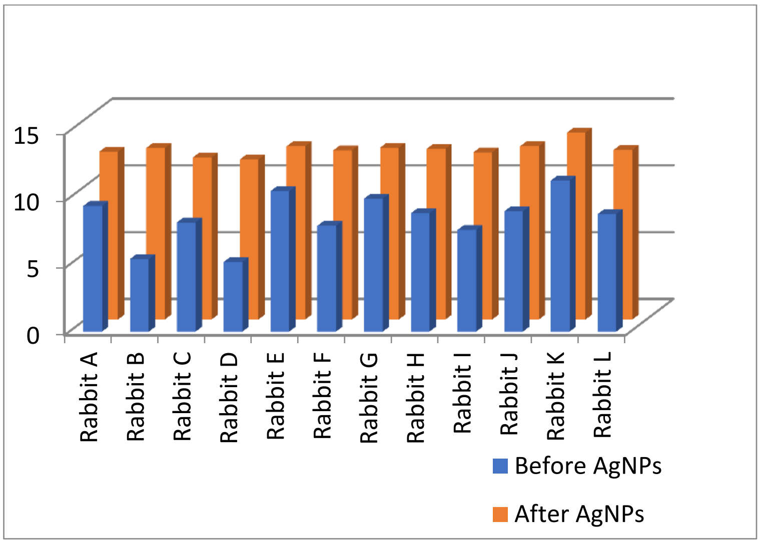

Fig 3- Bar graph showing WBC counts of rabbits before and after injection of silver nanoparticles.

immunity and induces T cells to produce various kinds of cytokinesLeukocytes are considered as the active cells in carrying out the functions of the immune system, both non-specific and specifically, and their count may give a general picture about the function of the immune system and the results demonstrated that a treatment with Ag-NPs influenced the differential count of leukocytes especially the Lymphocytes, Neutrophils, and Monocytes.
This increase in WBC count indicates that silver nanoparticles are recognized as infectious agents by the mammalian system and the immune system is triggered to produce white blood cells. This, on one hand, is the indication of the toxicological effects of silver nanoparticles [18], [11]and on the other hand, is also a property that can be exploited in cancer therapy, immunomodulation of vaccines and so on [16].

4.Conclusions and Recommendations

One of the most potential impacts silver nanoparticles can have is on medicine owing to their physicochemical characteristics, easily changeable morphologies, its interaction with various biological compounds and its effects on biological systems. There is a lot of scope for the silver nanoparticles in medicine with a need for a lot of future research to be conducted.
The future research should be focused on learning the detailed mechanism of interaction of silver nanoparticles with the mammalian system and its effects on immune system. Although silver nanoparticles have been identified as powerful antimicrobials, their possible toxicity limits their use as therapeutic agents. More detailed study on the nature of toxicity could lead to the identification of ways to minimize it. Modifying the silver nanoparticles with biological compounds would help in reducing the toxicity of the nanoparticles and in establishing silver nanoparticles as safe antimicrobials with certain

advantages over traditional antibiotics. The genetic mechanism controlling the interaction of a living system with silver nanoparticles can be further studies through gene expression analysis, thereby providing tools to manipulate the interaction to achieve maximum benefits.
More research should be conducted in studying the immunomodulatory behavior of the silver nanoparticles as this trait can be exploited in cancer therapy.
To conclude, our work helped in establishing silver nanoparticles as potentially toxic to mammals as well as immunomodulatory agents which could solve a lot of current problems in medicine

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