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Research Article

Influence of Metal Nanoparticles on Blood Coagulation

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Abstract:

Interaction of nanoparticles with the blood coagulation is important prior to their using as the drug carriers or therapeutic agents. The main aim of this research is to study the primary effects of silver nanoparticles (AgNPs) on haemostasis in vitro. Blood samples were obtained from Iraqi individuals (n=90) (32 females, 58 males; smokers 45 and non-smokers 45) aged between 25 to 65 years. The effect of AgNPs on blood coagulation was investigated directly estimating the activation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) and to verify any possible effect of AgNPs on human platelets.

In addition, the influence of various factors including health status, gender, and smoking activity on the values of PT and APTT levels in blood samples was also investigated. The results show that the values of PT and APTT were significantly increased by using 100 µg/ml of AgNPs. In contrast, there is no significant effect of health status, gender, and smoking activity on the level of PT and APTT at $P = 0.05$.

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1. Introduction

Silver nanoparticles (AgNPs) are known as nanomaterials in the dimensions range of 1–100 nm. It was found that the nanoparticles have presented greater capacity and higher surface (area-to-volume ratio) in comparison with those in bulk form [1]. These materials show unique optical, electrical, and catalytic properties at the nanoscale. This distinction led to the study and manufacture of products for targeted drug delivery, diagnosis, detection, and imaging [2]. In the last years, blood coagulation monitoring has become essential to identify the causes of hemorrhage, developing anticoagulant drugs, etc. [3]. The interactions between the nanoparticles and blood coagulation system can be useful or harmful depending on the suggested use of a nanomaterial. Nanoparticles can be organized to be procoagulant, anticoagulants; and delivery drug system in different pathological conditions in which coagulation is a concern [4,5]. Previous studies have reported that the use of nanoparticles as the drug carrier has become a major promising and it's an interest in scientists worldwide [6]. In addition, nanoparticles can be suggested as bacteriostatic agents [7]. Silver nanoparticles (AgNPs) have been historically used as an antimicrobial agent by itself or combined with other technologies to combat bacteria [1]. Moreover, AgNPs have been used in various biomedical applications, for example, they are as a novel delivery in tumor research vectors and as the carriers of anticancer drugs [6-10]. Furthermore, they can be used in several applications in the industrial area [13-16]. The aim of the present study is to investigate the estimation of the primary effects of AgNPs on haemostasis in vitro.

2. Materials and Methods

2.1 Materials

The $NaBH_4$ was obtained from Central Drug House (P) Ltd – CDH, and $AgNO_3$ was obtained from Thomas Baker.

2.2 Preparation of Metal Nanoparticles

In this procedure, 200 µL of Carbon Dots (CDs) were added to the solution of (9.8 ml) distilled deionized water including 1 mM of $AgNO_3$. Then, the solutions were left unstirred for 10 hrs. at room temperature. The formation of NPs confirms when the color of the solution is changed.

2.3 Preparation of Blood Samples

In this study, blood samples were taken from ninety people (healthy and patients) (n=90) from Kerbala, and their ages ranged from 25 - 65 years, as shown in Table 2.3. All samples were collected in the Teaching Al-Handia Hospital, laboratory of diagnosis, Karbala, Iraq. At least 2 mL of peripheral blood samples were collected using 3.2% sodium citrate tubes to prevent the clotting process from starting before the test [17]. Blood samples were centrifugated at 6000 rpm for 15 minutes in order to separate blood cells. Then, the mixture is left at 37°C for the period of 1-2 min. Excess quantities of ionized calcium were added to the mixtures to reduce the sodium citrate and allow clotting to start. Samples were analyzed using a Thrombotimer 4-channel instrument to determine the values of Prothrombin time (PT) and Activated Partial Thromboplastin Time (aPTT). A plasma sample without NPs was used as a control. Neoplastin CI plus 5 kit was used to determine PT, while C.K. pres. 2 reagent calcium (50 µL) was used to measure aPTT [18].

2.4 Instrumentations

Clotting time was monitored by the auto-start system Coagulation analyzer Thrombotimer 4-Channel [19].

2.5 Statistic Data Analysis

Statistical data analysis was performed using Microsoft Excel. All assays were performed in a series of three replicates. The values of arithmetic mean, standard deviation, paired t-test, and two-tailed t-test have been determined in this study [20].

3. Results and Discussion

3.1 Influence of AgNPs Concentration

The influence of AgNPs concentration on the values of PT and APTT was investigated. The results show that the higher effect of the concentration of AgNPs on the PT and APTT was 100 µg/ml, as shown in Figures 1 and 2. However, higher values of PT (13.40) and APTT (42.28) are found by using 100 µg/ml of AgNPs compared to other concentrations. Therefore, this concentration was used for future studies [21].

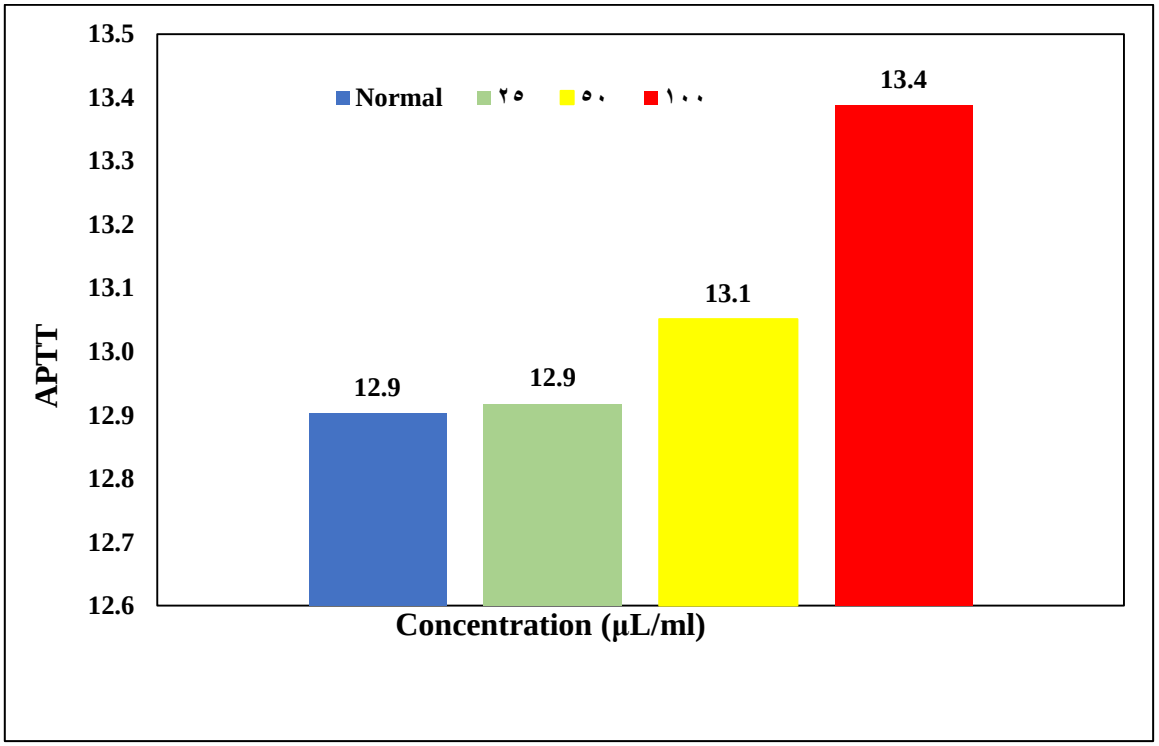

Figure 1: The effect of AgNPs concentration on the PTs (n=90).

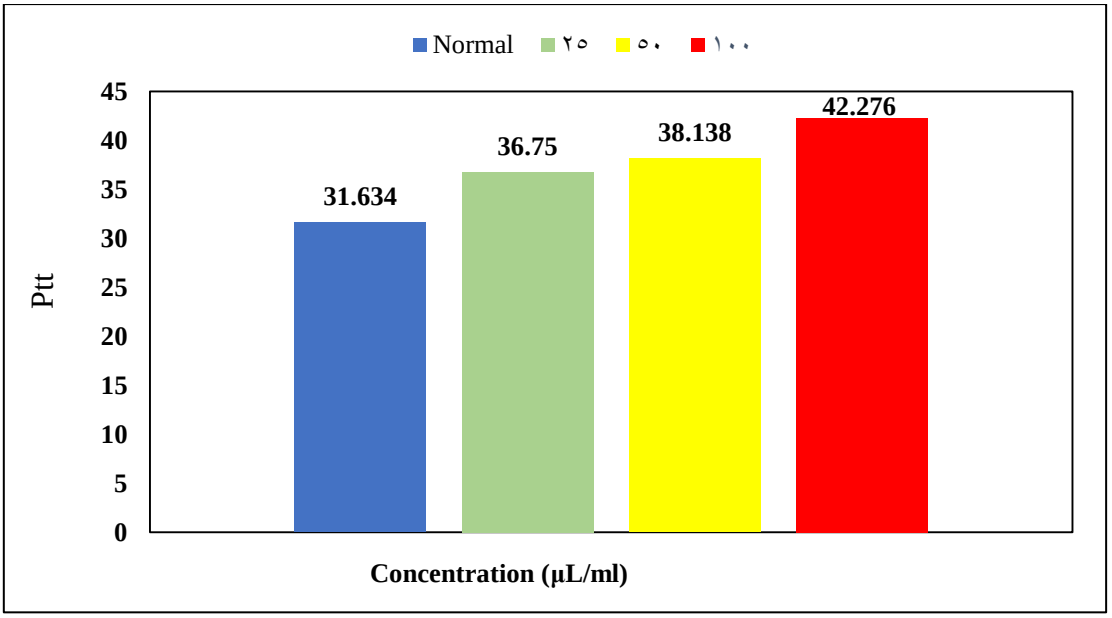

Figure 2: The effect of AgNPs concentration on the APTTs (n=90).

The values of mean and standard deviation of PT and APTT in blood samples with and without AgNPs are determined by using a paired t-test, and the results obtained are listed in Table 1. In general, significantly higher values of PT and APTT are found with AgNPs when compared with those without AgNPs, at the probability value of $P = 0.05$ [22].

Table 1: PT and PTT mean and standard deviation values in human blood for individuals with and without AgNPs from Karbala, Iraq.

	Mean (Unit)		(X – Y) Mean ± Sd (Unit)	Paired t-test			
	Without AgNPs (X) (n=90)	With AgNPs (Y) (n=90)		df	t_{calc}	t_{crit}	*Sig.(P)*
PT	12.902 ± 1.69	13.388 ± 1.74	0.486 ± 0.791	89	**5.829**	1.987	0.00
PTT	31.634 ± 6.68	42.276 ± 9.39	10.641 ± 6.092	89	**16.571**	1.987	0.00

Sd is the standard deviation, n_1, n_2 are the number of samples for individuals without and with AgNPs from Karbala, respectively, *df* = degree of freedom, $n_1 - 1$ for t-test, as described in Appendix C, t_{calc} are the calculated values for t-test, t_{crit} is a critical value at $P = 0.05$, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance.

3.2 Effect of Health Status.

The influence of health status on the values of PT and APTT levels in blood samples are reported in Table 2 using a two-tailed t-test. No significant effects were found in the health status for both of PT and APTT at P = 0.05. The findings agree with those found in the literature [23]. On the other hand, one study in Najaf, Iraq, has found significant differences in PT and APTT between hypertensive and normotensive patients [24].

Table 2: PT and PTT mean and standard deviation values in human blood for healthy individuals and patients from Karbala, Iraq.

	Mean ± SD (Unit)		Two-tailed t-test			
	Healthy ($n_1 = 30$)	Patients ($n_2 = 60$)	*df*	t_{calc}	t_{crit}	*Sig.(P)*
PT	12.640 ± 1.248	13.033 ± 1.870	88	1.039	2.021	0.301
PTT	30.020 ± 4.255	32.442 ± 7.512	88	1.636	2.021	0.105

Sd is the standard deviation, n_1, n_2 are the number of samples for health and patients, respectively, *df* = (n_1+n_2-2), the degree of freedom for t-test determined as described in Appendix C, and t_{calc} are the calculated values for t-test, t_{crit} is a critical value at $P = 0.05$, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance.

3.3 Influence of Gender

The purpose of this research is to determine whether PT and APTT levels differ between males and females [25]. Therefore, the influence of gender on the values of Pt and APTT in the blood samples was determined. The samples (n = 90) are divided into two groups, namely, females. The values of mean and standard deviation (±SD) for each group are presented in Table 3. The highest mean values in both groups are identified for APTT (male: 31.525 ± 7.505; and females: 31.842 ± 4.841). PT displayed the lowest values for both groups (males: 12.898 ± 1.906) and (females: 12.910 ± 1.207). The findings present that the PT and APTT levels of females were slightly higher

compared to males, but there is no significant effect of gender on the values of PT and APTT.

Table 3: PT and PTT mean and standard deviation values in human blood for males and females from Karbala, Iraq.

	Mean ± SD (Unit)		Two-tailed t-test			
	Male (n_1 = 59)	Female (n_2 = 31)	*df*	t_{calc}	t_{crit}	*Sig.(P)*
PT	12.898 ± 1.906	12.910 ± 1.207	88	0.032	2.021	0.975
PTT	31.525 ± 7.505	31.842 ± 4.841	88	0.213	2.021	0.832

Sd is the standard deviation, n_1, n_2 are the number of samples for males and females, respectively, $df = (n_1+n_2-2)$, the degree of freedom for the t-test determined as described in Appendix C, and t_{calc} are the calculated values for t-test, t_{crit} is a critical value at $P = 0.05$, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance.

3.4 Influence of Smoking Activity

The purpose of this study is to determine whether PT and APTT levels differ between smokers and non-smokers individuals [26]. Therefore, the effect of smoking activity on the levels of PT and APTT in has been studied and the results were reported in Table 4. It was found that there is no significant effect of smoking activity on PT and APTT in spite of the values of PT and APTT values are slightly higher in smokers compared to non-smokers.

Table 4.4: PT and PTT mean and standard deviation values in human blood for smokers and non-smokers from Karbala, Iraq.

	Mean ± SD (Unit)		Two-tailed t-test			
	Smokers (n_1 = 45)	Non-smoker (n_2 = 45)	*df*	t_{calc}	t_{crit}	*Sig.(P)*
PT	13.176 ± 2.098	12.629 ± 1.109	88	1.546	2.021	0.126
PTT	32.604 ± 8.021	30.664 ± 4.899	88	1.385	2.021	0.169

Sd is the standard deviation, n_1, n_2 are the number of samples for smokers and non-smokers, respectively, $df = (n_1+n_2-2)$, degree of freedom for t-test determined as described in Appendix C, and t_{calc} are the calculated values for the t-test, t_{crit} is a critical value at $P = 0.05$, the bold values indicate significant differences at the level of significance $P = 0.05$, Sig. = level of significance.

4. Conclusions

The AgNPs can increase the values of APTT and PT in blood samples. The possibility of AgNPs usage in nanomedicine is strongly dependent on their final concentration in the bloodstream and the size of the particles that are used.

5. Conflicts of Interest

The authors confirm that there are no conflicts of interest regarding the publication of this article.

6. Acknowledgement

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