Effect of zinc oxide nanoparticles on total salivary peroxidase activity of human saliva (In vitro study)

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ABSTRACT

Background: The potential use of zinc oxide and other metal oxide nanoparticles in biomedical are gaining interest in the scientific and medical communities, largely due to the physical and chemical properties of these nanomaterials. The present work revealed the effect of zinc oxide nanoparticles (ZnONPs) on the total salivary peroxidase enzyme activity of human saliva in comparison to de-ionized water.

Materials and methods: Forty eight unstimulated saliva samples were collected from dental students/University of Baghdad 18-22 years. Then measure the total salivary peroxidase activity first without any addition to human saliva as a control, second with dilution the saliva with de-ionized water, and third with zinc oxide nanoparticles in concentration (5.8 mg/ml).

Results: The results showed that there was significant inhibition of the activity of the total salivary peroxidase enzyme in the presence ZnO NPs and non-significant inhibition of enzyme activity in the presence de-ionized as compared with control group.

Conclusion: Zinc oxide nanoparticles have inhibition effect on total salivary peroxidase activity

Key words: Total Salivary Peroxidase activity, ZnO NPs, Human saliva. (J Bagh Coll Dentistry 2015; 27(2):178-182).

INTRODUCTION

Nanoparticles have many different effects on human health relative to bulk material from which they are produced [(1)].Nanotechnology has attracted global attention because nanoparticles (NPs) have properties unique from their bulk equivalents. NPs of Ag, CuO and ZnO are being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics and paints [(2)]. In recent years ZnO has received considerable attention because of its unique optical, piezoelectric, and magnetic properties [(3)].

ZnO NPs has been reported to have extremely good safety profile and no toxicity observed when taken at different nano sizes of the zinc particles [(4)]. Nanoparticles have large specific surface areas for adequate protein binding and biological interactions [(5)]; this revealed the great effect of ZnONPs on total salivary peroxidase activity. Zinc oxide nanoparticles (ZnO NPs), is an inorganic white powder and is insoluble in water [(6)].

ZnO is present in the earth crust as a mineral zincite; however, most ZnO used commercially is produced synthetically. ZnO is nontoxic and is compatible with human skin making it a suitable additive for textiles and surfaces that come in contact with human body [(7)]. It is well known that many antimicrobial proteins in saliva interact in vitrowith each other.

The interactions result in additive, synergistic, or inhibitory effects on Mutans streptococci, *lactobacilli*, or fungi. The main oral innatedefense factors are the peroxidase systems, lysozyme, lactoferrin, and histatins [(8)]. Peroxidase is an enzyme secreted from mammary, salivary, and other mucosal glands [(9)] that functions as a natural antibacterial agent [(10)]. Nanoparticles (NPs) have some advantages over small organic molecules. First, NPs have large specific surface areas for adequate protein binding and biological interactions [(11)]. Second, NPs can enter cells easily [(12)], in contrast to some small molecules and biological molecules. Third, there has been considerable progress in the synthesis of NPs with well controlled dimensions, geometry, and surface properties [(13)], to complement the structural complexity of proteins [(14)]. Recent developments in nanomaterials offer a new pathway for controlling protein behavior through surface interactions.The enzyme was less stable on nanoparticles surfaces than in free solution, and the stability was decreased further on larger particles with smaller surface curvature.Although the protein may retain most of its native structure after adsorption on the NPs surface. In some cases the thermodynamic stability of the protein is decreased, making the protein more sensitive to chemical denaturants such as urea [(15)].

MATERIALS AND METHODS

Zinc oxide nanoparticles provided from ministry of sciences and technology, with concentration 5.8 mg/ml for stock solution and the particles size >50 nm papered by sol gel method.

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We make different concentration from the stock solution by using dilution low (NV = NV).To confirm the activity of zinc oxide nanoparticles solution we make the UV Visspectra of ZnO NPs was shown in (Figure1). The absorption peak of the pre1pared ZnO NPs was found at around 400-500 nm.

Collection of Saliva Samples

Unstimulated (resting) whole saliva samples were collected (fromforty eight dental students Collage of Dentistry / University of Baghdad) under resting conditions between 8.0-11.0 A.M.

Students were asked to rinse their mouth with water and to generate saliva in their mouth and to spit into a wide test tube. The collection period was twenty minutes. Following the collection, the saliva was centrifuged at (2000 rpm) for 10 minutes. The resulting supernatant was stored at– 20 °C in polyethylene tubes until assayed.

Determination of Total Salivary Peroxidase activity

Salivary peroxidase activity was determined colorimetrically. Wide variety of hydrogen donors have been utilized in peroxidase assay systems. In this study an improved assay was adopted using 4- aminoantipyrine as hydrogen donor [16]. The activity is determined by measuring the increase in absorbance at = 510 nm resulting from the decomposition of hydrogen peroxide per time of incubation (ΔA/min).

Reagents

1. Phosphate Buffer (0.2 M) pH 7.0

a- A weight of 2.72 gm of KH2PO4 was dissolved in 100 ml de-ionizedwater.

b- A weight of 3.48 gm of K2HPO4 was dissolved in 100ml of de-ionized water. 60 ml of solution **b** is adjusted to pH 7.0 by adding appropriate amount of solution **a**.

2. Hydrogen Peroxide (0.0017 M)

This solution was prepared by diluting 1 ml of 30% hydrogen peroxide to 100 ml with de-ionized water; further dilution was carried out where 1 ml of this was diluted to 50 ml with (0.2M) potassium phosphate buffer (PH 7.0). This solution was prepared fresh daily.

3. 4-Aminoantipyrine (0.0025 M) with phenol (0.17 M)

This solution was prepared by dissolving 0.810 gm phenol in 40 ml de-ionized water, and then 0.025 gm of 4-aminoantipyrine was added, and diluted to a final volume of 50 ml with de-ionizeds solution should be kept in a brown bottle.

Procedure for Determination of TSP activity [16].

1- The following solutions were pipetted into test tube:

Solution	Test tube
Phenol / 4-aminoantipyrine solution	1.4 ml
0.0017M hydrogen peroxide	1.5 ml

2- The test tube was incubated at 25C° for 3-4 min. to achieve temperature equilibration.

3-The reaction was initiated by the addition of (0.1ml) of the sample (saliva), with mixing. The increase in the absorbance at wave length λ = 510 nm, was recorded for 5 minutes, to obtain ΔA/min.

Calculations of TSP Enzyme Activity

Difference in absorption per unit time (ΔA/min) was calculated, sinceΔA is the difference in absorbance between zero time and 5 minutes. One unit represents the decomposition of one µmole of hydrogen peroxide per min. at 25˚C and pH = 7 under the specified conditions according to this equation [17]:

Peroxidase activityU/L =

$$\frac{\Delta A/min}{\varepsilon} * \frac{Vt}{Vs} * 10^{6}$$

Where:-

Vt = total volume(3 ml)

Vs = sample volume(0.1ml)

ΔA/min = (Abs.at 5 min – Abs. at the zero time)/incubation time (5min.)

ε= Extiniction coefficient of phenol (50.000 L/mol/cm)

U/L = µmol / minute /liter of the sample.

Effect of ZnO NPs on Total Salivary Peroxidase Enzyme Activity

A stock solution (5.8 mg/ml) concentration of zinc oxide nanoparticles. The concentration of the nanoparticles are prepared by diluting with saliva using the stock solution. Total salivary peroxidase activity is measured in human saliva by using the same method with replace the ratio of 100% of saliva for the control by ratio70% saliva and 30% ZnO NPs solution from the stock solution only. So the final concentration of ZnO NPs solution became as fellow:

5.8 * 70/100 = 4.06 mg/ml.

While for diluting by de-ionized water we use 70% saliva and 30% de-ionized water.

RESULTS

One of the groups (Saliva + ZnO NPs) for the total salivary peroxidase enzyme activity is not normally distributed significance $p < 0.05$ by Kolmogorov-Smirnov test; this revealed a lack of normal distribution of data. Therefore, non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for data analysis with SPSS 14 statistical software at a 0.05 Significance level.test for test of normality (Table 1).

Descriptive statistics for the total salivary peroxidase activity measured in U/L used to examine the differences among three different groups of total salivary peroxidase activity (Table 2), each group consist of 48 tests for Saliva + De-ionized water group, Saliva+ ZnO NPs group and 18 tests for the control group, while the (Figure 2) show only mean and SD of inhibition zones for three different groups. Results of Kruskal-Wallis test showed highly significant differences among the three groups $p< 0.01$(Table3).

Further analysis using a Mann-W hitney U Test was done to determine which of the three study groups was different from the other groups. The results of Mann-Whitney U test for the three groups showed highly significant $P< 0.01$ between (Control group- ZnO NPs group) and between (De-ionized water group- ZnO NPs group), while for the (Control group - De-ionized water group) show no significance $P > 0.05$ as shown in (Table 4).

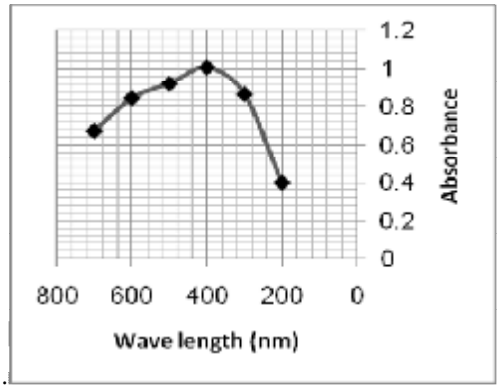

Figure 1: UV-Vis spectra of the ZnO NPs

Table 1: Test of Normality of groups of Total Salivary Peroxidase Enzyme Activity

Kolmogorov-Smirnov Test		
	Groups	Sig.
Activity	**Control (saliva only)**	0.168
	Saliva +De-ionized water	0.200
	Saliva+ ZnO NPs	0.034

Table 2: Descriptive statistics for the total salivary peroxidase activity in U/L

Groups	Median	Mean	No.	SD	Max.	Min.	Interquartile- range
Control(saliva only)	6.24	6.66	18	2.02	10.44	3.84	3.51
Saliva + De-ionized water	5.88	5.55	48	2.02	8.76	1.92	2.73
Saliva+ ZnO NPs	0.84	0.92	48	0.48	1.68	0.12	0.84

Table 3: Effect of ZnO NPs on TSP activity

	Groups	N	Mean Rank	Kruskal-Wallis H Test
Activity	**Control (saliva only)**	18	88.06	Chi-Square= 83.702
	Saliva + De-ionized water	48	79.04	df= 2
	Saliva + ZnO NPs	48	24.50	P <0.01HS

Table 4: Mann-Whitney U Test among different three groups

Groups	Median	Mean Rank	U Value	ZValue	P Value	Sig.
Control (saliva only)	6.24	40.06	314.00	1.70	0.09	NS
Saliva + De-ionized water	5.88	31.04				
Control (saliva only)	6.24	57.50	0.00	6.23	< 0.01	HS
Saliva+ ZnO NPs	0.84	24.50				
Saliva + De-ionized water	5.88	72.50	0.00	8.45	< 0.01	HS
Saliva+ ZnO NPs	0.84	24.50				

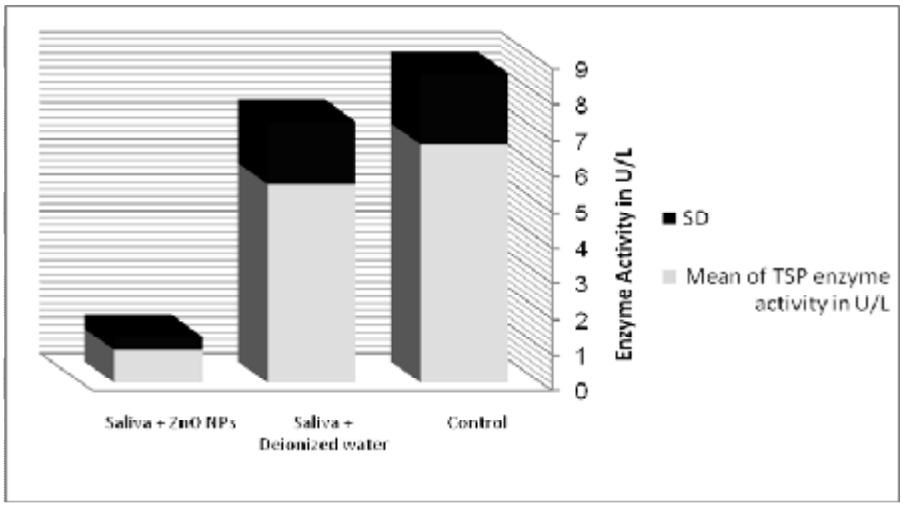

Figure 2: Effect of ZnO NPs on total salivary peroxidase activity

DISCUSSION

Kolmogorov-Smirnov test revealed a lack of normal distribution of data. Therefore, non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for data analysis with SPSS 14 statistical software at a 0.05 Significance level.The results of effect of zinc oxide nanoparticles on total salivary peroxidase activity shown in figure (2) and table (4).

The activity of the enzyme in saliva only without any addition was considered as control and was equal to (6.66 ± 2.02U/L) while other results showed difference in the control value with the same units, the same procedure and same condition from pH and temperature, the normal value of total salivary peroxidase enzyme in control group was equal to (24.24± 18.85). This reflects high difference of enzyme activity in saliva that may affected by any variation in procedure [16,17].For the comparison of control (which contain saliva only) and de-ionized watergroup (which contain saliva70% and 30% deionized water) no significant decrease in comparison with the control (P>0.05) with decreasing the activity to (5.55± 2.02) these results may be attributed to dilution of saliva by de-ionized water.

The biochemical tests revealed that ZnO NPs in concentration of 30% saliva and 70% ZnO NPs from the stock (5.8mg/ml) which is equal to (4.06 mg/ml) solution caused inhibitory effects on total salivary peroxidase enzymes activities, as shown in (Figures 4). This inhibition may be attributed to heavy metals which are toxic and react with proteins, therefore they bind protein molecules. Heavy metals strongly interact with thiol groups of vital enzymes and inactivate them. These results agree with that of Chudasama et al. [18]. In addition, it is believed that Zn bind to functional groups of proteins, resulting in protein deactivation and denaturation. This fact strongly agrees with other results [19,20].

The present study hypothesizes that ZnO NPs interact with functional groups of TSP enzymes, resulting in protein denaturation and inactivate it, and as a conclusion, the ZnONPs inhibited the enzyme. Our work could not be compared to other work exactly because as far as we know this is the only study that demonstrates the effects of zinc oxide nanoparticles solution on the activities of total salivary peroxidase enzyme activity.

As conclusion; Zinc oxide nanoparticles have inhibition effect on total salivary peroxidase activity in comparison to de-ionized water.

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