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Research Article

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Estimation of ZnO Nanoparticles Effect on Salivary ALP Activity in Chronic Periodontitis Patients: *in vitro* study

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Abstract

Background: Medical applications of nanoparticles **(**NPs**)** have been studied in different areas to estimate the effect of these nanoparticles on the studied parameters. Evaluation of the effect for any type of these nanoparticles can be achieved in terms of the relevant biochemical parameters of the case under study. In this work, the effect of ZnO NPs on salivary alkaline phosphate (ALP) in patients with chronic periodontitis was studied. **Materials and Methods:** saliva samples of 60 persons, aged (30-60) years with chronic periodontitis were collected as patients group**,** as well as samples of saliva were obtained from 20 healthy subjects, aged (30-60) as control group. Powder of ZnO NPs (<80 nm) was used in this study. Salivary ALP activity was estimated in patients group(without NPs) as a relevant biomarker of chronic periodontitis. Effect of ZnO NPs on chronic periodontitis was evaluated in terms of ALP activity in saliva of patients group with NPs. ALP activity was estimated by colorimetric method. **Results**: results were showed that ALP activity in patients group without ZnO NPs was higher than its activity in control group. While, the activity of ALP was decreased in patients group with ZnO NPs related to patients group without NPs. ALP activity in both patient groups (with and without NPs) was higher than its activity in control group. **Conclusion**: The effect of ZnO NPs on salivary ALP activity may be attributed to the vital role of ZnO NPs in resistance of the pathogens, in another hand, this effect may be reflects the conformational changes on protein structure after interaction with ZnO NPs.

Keywords: ALP, Saliva, ZnONPs and chronic periodontitis.

Introduction

Nanoparticles have a greater surface area per weight in compared to the large particles. This property renders the result nanoparticles more active powder [1].Recently, effect of nanoparticles of gold, silver and TiO2 NPs on salivary acid phosphatesACP, alkaline phosphates ALP and LDH was evaluated [2-4]. Recent studies found that nanoparticles of zinc oxide possess biological activity toward some types of pathogenic bacteria [5-7]. As it was known, the periodontal diseases are bacterial infections of the gingiva and other attachment fibers that support the teeth. [8].

Zinc oxide nanoparticles are found to be nontoxic, biosafe, and biocompatible and have been used as drug carriers, cosmetics, and fillers in medical materials. On the other hand, most ZnO nano-particles that used commercially have some advantages, compared to silver nano-particle, such as lower cost and white appearance [9].

Many material products including lubricants, paints, ointments, adhesives, pigments and fire retardants contain zinc oxide powder as an additive substance [10].

Alkaline phosphatase ALP is classified as a hydrolytic enzyme. ALP responsible for removing phosphate group from many types of biomolecules such as proteins, nucleotides and alkaloids, through dephosphorylation process. It possesses a high activity at alkaline medium, so sometimes called as basic phosphatase. [11].The principal source of ALP includes polymorphonuclear leukocytes (PMN), bacteria within the supra and subgingival plaque and through fibroblast and osteoblast activity. It is formed by many cells within the periodontal environment, so ALP considers potentially powerful markers of periodontal disease activity [12].

ALP plays a principal role in bone homeostasis. It is found in various organs such as liver, kidney, bones, intestine and placenta, also it presents in different cells of periodontium including neutrophils, osteoblasts and fibroblasts. Neutrophils, osteoblasts and ligament fibroblast are releasing ALP during bone formation and periodontal regeneration respectively. Consequently it has double participation in periodontal inflammation process andcuring or restoration. There are diverse sources of potential sample to estimate ALP including, include Gingival crevicular fluid (GCF), saliva and serum [13]. Recently, saliva compounds study shows a correlation between some of the biochemical markers like alkaline phosphatase, Glucoronidase, Immunoglobulins (IgA, IgG), hormones and their relation to the severity of periodontitis. Thus, saliva considers an important diagnostic fluid in medicine and dentistry [14]

The aim of this study is to evaluate the effect of zinc oxide nanoparticles on salivary ALP in patients with chronic periodontitis together with the estimation of its activity in patient's saliva without nanoparticles as a relevant biochemical parameter of chronic periodontitis. .

Materials and Methods

1- Nanoparticles

Zinc oxide nanoparticles have been obtained from Nanjing, china. This product supplies as ZnO Nano powder absorbance spectra of NPs stock solution were measured by UV- VIS spectrophptometer . Structure and nano size measurement of ZnO NPs powder were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre-College of applied Science, University of Technology, Iraq).

2- Collection of Saliva:

Un-stimulated whole saliva was collected after the clinical examination. A sample was collected after an individual was asked to rinse his mouth thoroughly with water to insure the removal of any possible debris or contaminating materials and waiting for 1-3 min for water clearance. The samples were collected at least 1 h after the last meal. Saliva was collected between 9-11 a.m. Each one of the groups' subjects was asked to spit saliva into the polyethylene tubes until 5 ml was collected. Samples containing blood were discarded. Then the container was labeled with the number of the subject and kept in the cooling box. Then the collected saliva was separated by centrifuge at 4000 rpm for 10 minutes and then the clear supernatant saliva divided into 3 parts by micropipette into eppendorf tubes and store at -20˚C (freeze) until biochemical analysis.

3- Salivary ALP assay:

In a test tube 100µl of saliva sample was added, then 1000µl of Diethanolamine buffer (pH 10.35± 0.2) (1.25 M) added, mixed and incubated for 1 min. at room temperature. Then, 250µl of substrate solution p-Nitrophenyl phosphate (50 mmol/L) was added and mixed. The absorbance at 405 nm was read after 1 min. again after 3 minutes.

4- Effect of ZnO nanoparticles on salivary Total ALP activity:

Salivary alkaline phosphatase activity was determined by kinetic method. Stock solution of (300 µg/ml) concentration of ZnO NPs was prepared and then the following concentrations (5, 10, 20, 40, 80, and 100) µg/ml have been prepared by diluting with the same solvent. ALP activity was measured in human saliva by using 100 µl of saliva in the same method with replace 20 µl of the solvent (3:1, water:ethanol) with 20 µl of ZnO NPs solution. And then salivary ALP activities were determined in control group.

The percentage effect on activity was calculated by comparing the activity with and without ZnO NPs and under the same conditions according to the following equations:

٪ inhibition = 100 – 100 x (Activity in the presence of nanoparticles / Activity without nanoparticles) .

A constantfinal concentration of ZnO NPs (18.25µg/L) was used to measure the enzyme activity in saliva samples of chronic periodontitis patients.

5-Statistical analysis:

Data were analyzed using SPSS software version 19. Descriptive statistics including medians, means, standard deviations, minimum, maximum values and inferential statistics including Kruskal -Wallis H test, Mann-Whitney U test, Spearman´s rank correlation coefficient test (r) were used in this study. Values of P> 0.05, 0.05 ≥ P > 0.01, P≤ 0.01 were considered non- significant (NS), significant (S) and highly significant (HS) respectively.

Results and Discussion

ALP activity in chronic periodontitis:

The Table (1) shows mean and standard deviation of ALP of control group (0.470 ± 0.268) and patients group (1.145 ± 0.477). The results revealed high significant difference between control and chronic periodontitis patients, correlations between ALP and clinical periodontal parameters (PLI and GI) in control group were found to be weak non- significant negative correlations as shown in table (2). While the table (3) shows a weak non-significant correlations were revealed between ALP activity and PLI, GI, pocket depth and bleeding of scored (0) , and a weak non-significant negative correlations shown in clinical attachment level , bleeding of scored (1) in chronic periodontitis patients .

Table (1): Descriptive statistics and significant differences of AL Pactivityin control and chronic periodontitis groups

Groups	Descriptive statistics						
	N	Median	Mean	S.D.	Min.	Max.	p-value
ALP Activity in control	20	0.5	0.470	0.268	0.2	1	0.000 (HS)
ALP Activity in CP	60	1.1	1.145	0.477	0.5	1.9	

Table (2): Correlation between ALP and periodontal parameters in control

Control group		PLI	GI
activity of ALP (U\L)	R	-0.036	-0.321
	p-value	0.880 (NS)	0.167 (NS)

*Significant NS at (P>0.05) level of significance

Table (3): Correlation between ALP and periodontal parameters in C.P.

C.P group		PLI	GI	CAL	PPD	BOP% (0)	BOP% (1)
ALP activity with-out nano (U\L)	R	0.052	0.182	-0.221	0.156	0.062	-0.062
	p-value	0.692 (NS)	0.164 (NS)	0.090 (NS)	0.234 (NS)	0.639 (NS)	0.639 (NS)

*Significant NS at (P>0.05) level of significance

The results in present study are agreement with numerous recent studies which were referred to increase of ALP activity in patients with periodontal disease than its activity in healthy persons [15, 16]. At the same time, other studies were found non-significant correlation between ALP and clinical periodontal parameters [17-18]. In contrast, study conducted by Kumar R, Geeta Sharma was disagreement with our results [19].

UV- VIS absorption spectra:

Spectra of UV-VIS were indicated the characteristic absorbance feature of Zinc oxide nanoparticles , the maximum absorption peak of ZnO NPs, which suspended in ethanol-water mixture, was showed at 375 nm as shown in figure (1). This absorption peak considers as a hallmark of ZnO NPs at applied nanoparticles size (<80 nm).

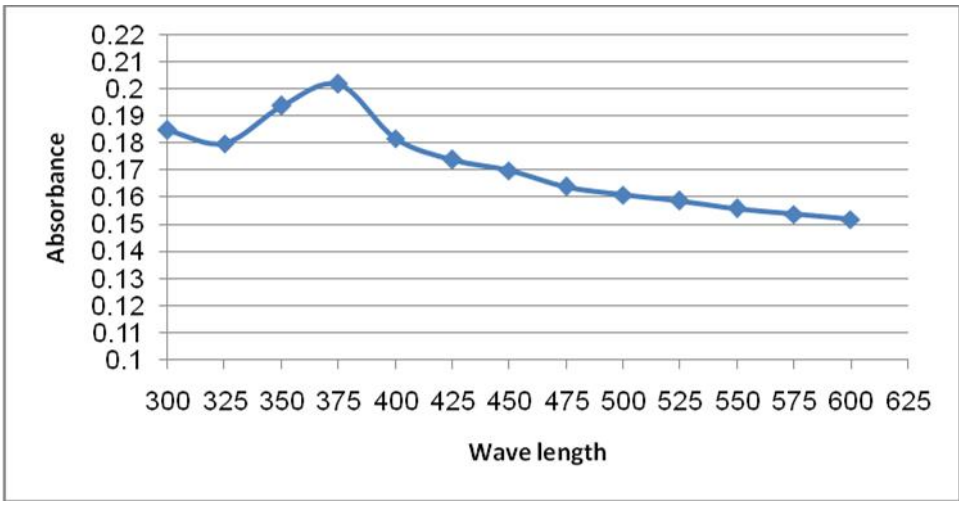

Figure (1): UV – VIS spectra of the ZnO NPs

Scanning Electron Microscope (SEM):

Figure (2) shows SEM pictures and size distributions of ZnO NPs using in this study. The average diameters of the produced NPs were found to be of <80nm.

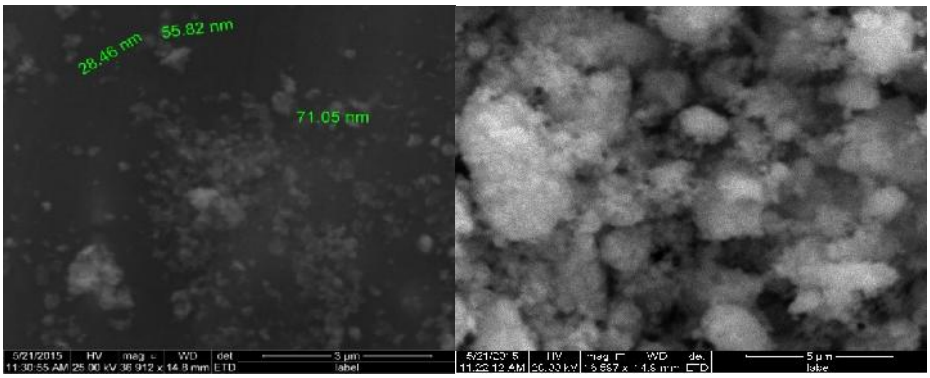

Figure (2): shows SEM picture and size distributions of ZnO NPs.

Effect of ZnO NPs on Salivary Alkaline phosphatase (ALP) Activity:

The activity of salivary ALP was measured in unit/liter for all the studied groups (control, patients without ZnO NPs and patients with ZnO NPs groups). The results of this effect were shown in table (3). ALP activity (mean ± SD) in presence of nanoparticles of zinc oxide (0.778 ±0.453) was less than its activity in patient's saliva without NPs (1.145 ± 0.477), at the same time enzyme activity in both patient groups (with and without ZnO NPs) was higher than its activity in control group. Highly significant difference was found to be among the studied groups (p=0.000). These results were illustrated in fig. (3), which was showed the effect of ZnO NPs on salivary ALP clearly.

Table (4): The median, mean and standard deviation for salivary ALP activity in chronic periodontitis patients with and without ZnO NPs and control groups.

Groups	Descriptive statistics						
	N	Median	Mean	S.D.	Min.	Max.	p-value
ALP activity in control group	20	0.5	0.470	0.268	0.2	1	0.000 (HS)
ALP activity without NPs group	60	1.1	1.145	0.477	0.5	1.9	
ALP activity with NPs group	60	0.7	0.778	0.453	0.2	1.7	

*Significant HS at (P≤ 0.01) level of significance

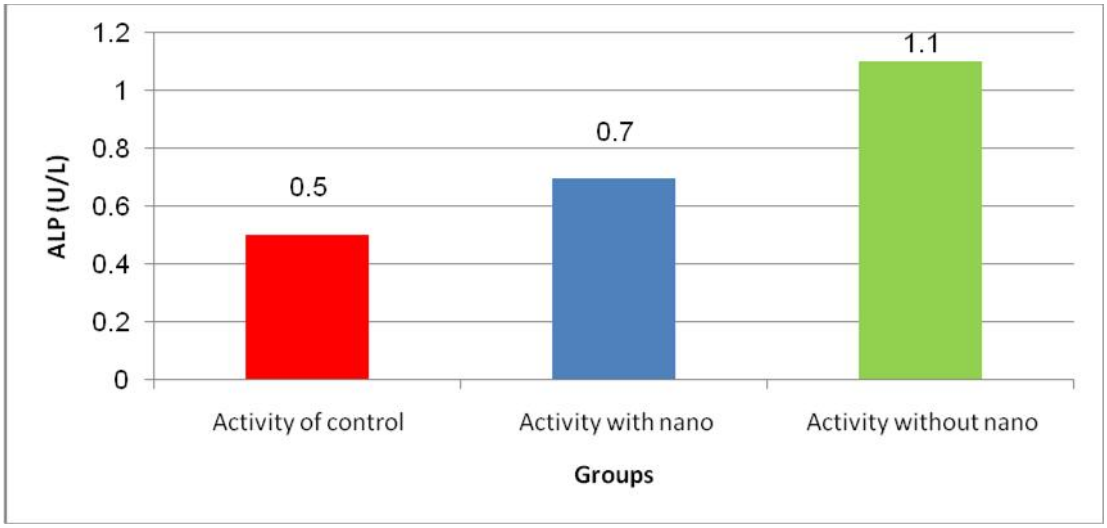

Figure (3): Median of ALP activity in patients with and without ZnO NPs and control group.

Further analysis using a Mann – Whitney U Test was conducted to determine the significant differences between any two groups of the mentioned groups in this study. The results revealed a highly significant differences between groups of control versus patients without nano (p= 0.009), control versus patients with nano (p=0.000) and patients with nano versus patients without nano (p=0.000) as shown in table (5).

Figure (4) shows the effect of ZnO NPs (µg/ml) on the activity of salivary ALP (U/L) in a total volume of reaction mixture (1370 µl). The greater inhibition of ZnO NPs on enzyme activity was found to be at concentration of 1.46µg/ml in total volume of the reaction mixture.

Table (5): Mann – Whitney U Test among different three groups of control, with and without ZnO NPs.

Groups / Statistics	Control vs. Patients with nano	Control vs. Patients without nano	Patients with nano vs. without nano
Mann-Whitney U	368	132	1060
p-value	0.009 (HS)	0.000 (HS)	0.000 (HS)

*Significant HS at (P≤ 0.01) level of significance

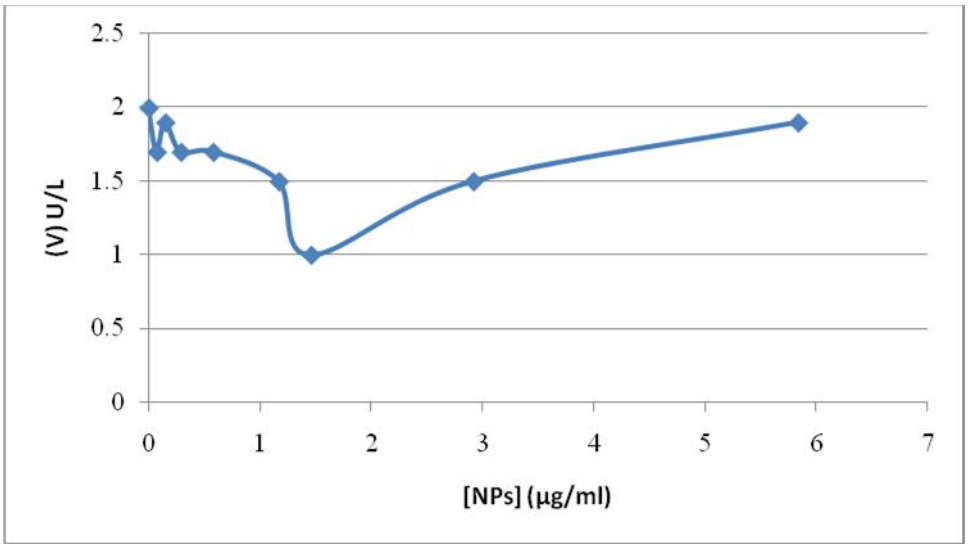

Figure (4): Salivary ALP activity in presence of different concentration of ZnO NPs.

The greater inhibition percentage of enzyme activity by ZnO NPs was found to be of 50٪ at concentration of 1.46µg/ml, as a more effective NPs concentration among others, as shown in figure (5).

The results of our study were referred to that the presence of ZnO NPs was lead to decrease the activity of ALP in abnormal case (1.145 ±0.477- 0.778 ±0.453) toward its activity in normal case (0.470± 0.268), these findings may be attributed to the vital role of ZnO NPs in resistance of the pathogens [5-7]. In contrast, more recent study revealed that ZnO NPs (1-5mg/ml) was increased the activities of ALT, AST, ALP and LDH in mouse myoblasts; the study suggested that the effect of ZnO NPs is a dose - dependent manner [20].

The results in this study is agree with more recent study [21], which found that TiO2 NPs inhibited salivary ALP in periodontitis patients greatly in (0.15µg/ml). In another study Al-Rubaee et al [22] were reported that both Au and Ag metal nanoparticles inhibited the activity of acid phosphatase activity in sera of healthy subjects, the greater inhibition of Au NPs on ACP activity was 5% at concentration (5.7)µg/ml and Ag nanoparticles was (5.8)% at concentration(10)µg/ml.

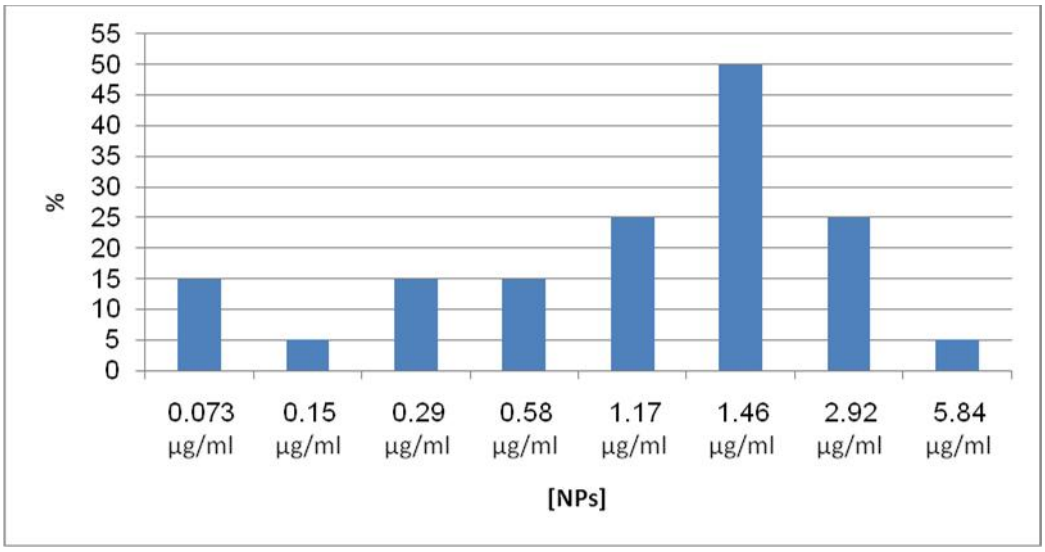

Figure (5): percentage inhibition of salivary ALP activity in different concentrations of ZnONPs.

Schug et al [23] showed that in intact heterotrophic biofilms, alkaline phosphatase activity was not affected following contact to surface functionalized TiO2 NPs and UV radiation. While, an alkaline phosphatase enzyme excreted from E. coli was strongly inhibited at lower concentration of ZnO NPs than the intact biofilms.

Heavy metals are toxic and react with proteins, therefore they bind protein molecules. Heavy metals strongly interact with thiol groups of vital enzymes and inactivate them [24]. In addition, it is believed that the metal NPs like Ag bind to functional groups of proteins, resulting in protein deactivation and denaturation [25, 26].

Conclusion

It can be concluded from the obtained results of this study that ZnO NPs inhibited the activity of salivary ALP in compare to its activity without NPs. This effect may be attributed to conformational changes on protein structure after interaction with ZnO NPs. In another hand, ZnONPs possess antibacterial activity that could be effect on pathogens and decline its abnormal activity toward normal value.

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