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Assessment of ZnO Nanoparticles Effect on AST Activity in Saliva of Patients with Chronic periodontitis: *in vitro* study

Kadhim K. Ghudhaib*, Leka´aM. Ibrahim **, Eaman A. Al-Rubaee*, Zainab A. Salman***

*Department of Chemistry, College of science for women, University of Baghdad, Baghdad, Iraq.
** Periodontal Department, College of Dentistry, University of Baghdad, Baghdad, Iraq.
***Department of Basic Science, College of Dentistry, University of Baghdad, Baghdad, Iraq.
*Corresponding Author:

Abstract

Background: nanoparticle applications have been evaluated in different areas of sciences, among these areas are the medical applications. Usually, the estimation of their effects in this field can be evaluated via establishing of their effects on the relevant biochemical functions of the studied disease. The aim of this study is to estimate the effect of ZnO nanoparticles on salivary AST activity in chronic periodontitis patients. **Materials and Methods**: saliva samples of 60 patients, aged (30-60) years, with chronic periodontitis were collected as a patients group. Control group included 20 samples of saliva were obtained from 20 healthy subjects, aged (30-60). Nanoparticles of ZnO were used in this study in a diameter less than 80 nm. Salivary AST activity was estimated in saliva of the studied groups in absence and presence of ZnO Nanoparticles by colorimetric method. **Results**: the obtained results in this study were showed that the activity of salivary AST in presence of ZnO NPs in patients group was higher than its activity in this group without ZnO NPs. at the same time, both its activities in patients group (with and without ZnO NPs) were found to be higher than its activity in control group. **Conclusion:** the results in this study show increasing of salivary activity in presence of ZnO NPs. this effect may be attributed to the biological activity of this type of NPs in addition to the conformational changes that can be occurred on the protein structure after interaction with NPs.

Keywords: Saliva, AST, Chronic periodontitis, ZnO NPs.

Introduction

Nanoparticles possessing a higher surface area than other large particle for the same origin, this property make them to become as active powder and can be used in many applications [1].More recent studies found that ZnO NPs inhibit activities of salivary alkaline phosphate (ALP) and salivary peroxidase in patients with chronic periodontitis [2-3]. Recent studies referred to present an effect of gold, silver and TiO_2 NPs on activities of acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) [4-6]. Biological activity of ZnO NPs was recorded against certain pathogenic Bactria in other recent studies [7-9].

Periodontitisis a chronic infection concerning destruction of the tooth-supporting apparatus, including theperiodontal ligament and alveolar socket support of the teeth. Gingivitis may or may not progress to periodontitis, which is related with accessoryand alveolar bone loss. Periodontal disease is introduced by a local accumulation of bacteria (i.e., dental plaqueadjacent to the tooth) and theirmetabolic products (e.g., endotoxin), that stimulate the junctional epithelium to proliferate and produce tissue-destructive proteinases [10].

Zinc oxide nanoparticle properties are including many good features among other nanoparticles such as biosafe, nontoxic and biocompatible, in addition to other applications including drug carriers, cosmetic and fillers in medical materials. Also, ZnO NPs possess some advantages include lower cost and white appearance commercially [11].

AST found in many body tissues especially in liver, heart, and skeletal muscle. AST serves as a diagnostic factor of cellular injury. It is a ubiquitous component of saliva and is detected in periodontal tissue, GCF, enamel pellicle, and saliva. Thus, AST can be a biochemical marker of the functional condition of periodontal tissues [12]. AST enzyme is indicator of a higher level of cellular damage and its increased activity in saliva is a result of its increased release from the damaged cells of soft tissues in the inflamed gingiva[13]. It was found that the AST enzyme levels were significantly elevated in saliva of patients with chronic periodontitis, with correlating to the tissue destruction taking place in these conditions. The importance has been given to AST activity in saliva for diagnostic purpose [14].

The aim of this study is to evaluate of the salivary AST in saliva of patients with chronic periodontitis as a relevant biomarker and to establish the effect of ZnO NPs on the activity of salivary AST in patients group in comparison with its activity in both patients group without ZnO NPs and control group.

Materials and Methods

1- Nanoparticles

Zinc oxide nanoparticles have been obtained from Nanjing, china. This product supplies as ZnO nano powder. UV-VIS spectrum of NPs stock solution was recorded by UV- VIS spectrophotometer. 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- Sample Description:

The study sample was consisted of eighty participants with age range of (30-60) years. Sample collection was started on March to June of 2015. The participants recruited for the study were patients who attending the Department of periodontic in the College of Dentistry, University of Baghdad. All participants were informed about the aims of the study orally and by written as a written informed consent was assigned by all participants.

All participants were subjected to a questionnaire about their names, ages, full medical history, medication, if they smoked or not and if there was any previous periodontal treatment. All subjects were presenting at least 20 teeth. Sample of whole unstimulated saliva was taken from each subject. Following this full examination of clinical periodontal parameters: Plaque Index (PLI), Gingival Index (GI), Bleeding on probing (BOP), Probing Pocket Depth (PPD) and clinical attachment level CAL) was done for all subjects. The exclusion criteria were including: A course of anti-inflammatory or antimicrobial therapy within the previous three months, a history of regular use of mouth washes, use of any vitamin supplementation, mucosal lesions, chemotherapy, radiation therapy, medications that cause xerostomia.The participants were divided into two groups include study group which is consisted of sixty participants with chronic periodontitis only without history of any systemic diseases.(patients with chronic periodontitis should have at least 4 sites with pocket depths ≥4mm with clinical attachment loss of (1-2)mm or greater ,this was measured according to[15,16], and control group which is consisted of twenty participants who were apparently healthy systemically and with clinically healthy periodontium.

3- Saliva samples collection

Un-stimulated whole saliva was collected before 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, the clear supernatant saliva divided into 3 parts by micropipette into eppendorf tubes and store at -20˚C (freeze) until biochemical analysis .

4- Salivary AST assay

Colorimetric method (Reitman and Frankel) was used to determine salivary aspartate aminotransferase

activity. The measurement was conducted by monitoring the concentration of oxaloacetate hydrazone formed from oxaloacetate with 2, 4 dinitrophenyl – hydrazine [17].

5- Effect of ZnO NPs on Salivary AST activity determination

Salivary aspartate aminotransferase was determined by colorimetric method. Stock solution of (300 µg/ml) concentration of ZnO NPs was prepared. The following concentrations (5, 10, 20, 40, 80, and 100) µg/ml were prepared by diluting with the same solvent. The enzyme activity was determine by using 100µl of saliva and 20µl of ZnO NPs solution, the same steps were conducted for another run without NPs to evaluate the effect of NPs on the enzyme activity by adding 20µl de-ionized water. The percentage ratio of activation on activity was calculated by comparing the activity with and without the ZnO NPs according to the following equation:

$$\text{activation\%} = 100 \frac{\text{Activity in the presence of nanoparticles}}{\text{Activity withcut the nanoparticles}} - 100$$

The final concentration of ZnO NPs (0.33µg/ml) was used to identify the enzyme activity in saliva samples of chronic periodontitis patients.

6- Statistical analysis

Analysis of data was achieved 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 $P > 0.05$, $0.05 \geq P > 0.01$, $P \leq 0.01$ were considered on- significant (NS), significant (S) and highly significant (HS) respectively.

Results and Discussion

AST activity in chronic periodontitis:

Table (1)showed the mean and standard deviation of AST activity for control group (0.420 ± 0.247)and in CP group the mean ± SD was (1.210 ± 0.702) , there was high significant difference between control and chronic periodontitis patients ,and the table (2) shows that a weak non significant negative correlation between AST activity and the clinical periodontal parameters (PLI and GI) in control group . While the table (3) shows a weak non significant correlation between AST activity and PLI, GI, pocket depth and bleeding of scored (1) , and a weak negative correlation was shown between the enzyme activity and the parameters, clinical attachment level , bleeding of scored (0) in chronic periodontitis patients. Non significant differences of AST activity in saliva for control as compared to chronic periodontitis patients.

Table (1): Descriptive analysis of AST in control and chronic periodontitis groups

Groups	Descriptive statistics						
	N	Median	Mean	S.D.	Min.	Max.	p-value
Activity in control	20	0.355	0.420	0.247	0.16	1.1	0.000 (HS)
Activity in CP	60	0.98	1.210	0.702	0.16	2.99	

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

Table (2): Correlation between AST activity and clinical periodontal parameters in control.

AST		PLI	GI
activity of control (U\L)	**R**	-0.121	-0.016
	p-value	0.611 (NS)	0.946 (NS)

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

Table (3): Correlation between AST activity and clinical periodontal parameters in C.P. group

AST		PLI	GI	CAL	PPD	BOP% (0)	BOP% (1)
activity without nano (U\L)	R	0.104	0.118	-0.090	0.064	-0.070	0.070
	p-value	0.429 (NS)	0.368 (NS)	0.494 (NS)	0.627 (NS)	0.595 (NS)	0.595 (NS)

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

The results obtained in the present study were in coordination with many other studies [18-20].Similarly, the levels of Aspartate aminotransferase enzyme in gingival crevicular fluid was increased in periodontitis patients in compare healthy and gingivitis patients as it reported elsewhere [21, 22].Luke et al [23] were found that highly significant difference of AST activity in saliva between CP patients and control groups. In Another hand, Totan et al [20] was estimated the level of AST in saliva from CP patients and agreement with this study.

Popovic et al [24] agreement with this study, who found that non –significant correlation between AST activity and clinical parameter. In another study [25], it was reported that there was non significant correlation between AST activity and clinical periodontal parameters. Wahab and Ahmed [26] disagreement with this study, they found that there was highly significant strong positive correlation between salivary AST activity and CAL in CP patients. The intracellular of AST activity included in the metabolic processes of cells and they were indicators of a higher level of cellular distraction and a reflection of metabolic change in the inflamed gingival tissue [27-29].

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).

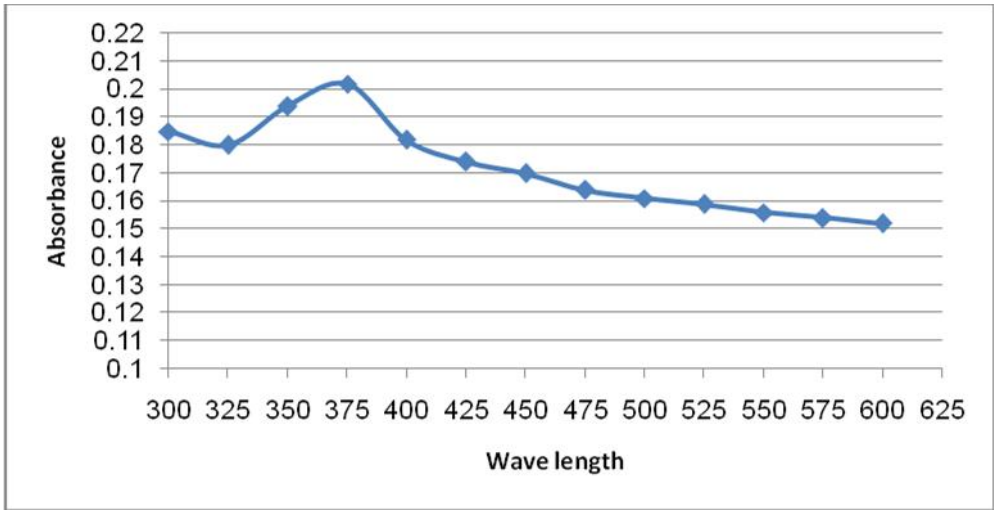

Figure (1): UV – VIS spectrum of the ZnO NPs

Scanning Electron Microscope (SEM)

Figure (2) shows SEM picture and size distribution of ZnO NPs using in this study. The average diameters of the produced NPs were found to be less than 80nm.

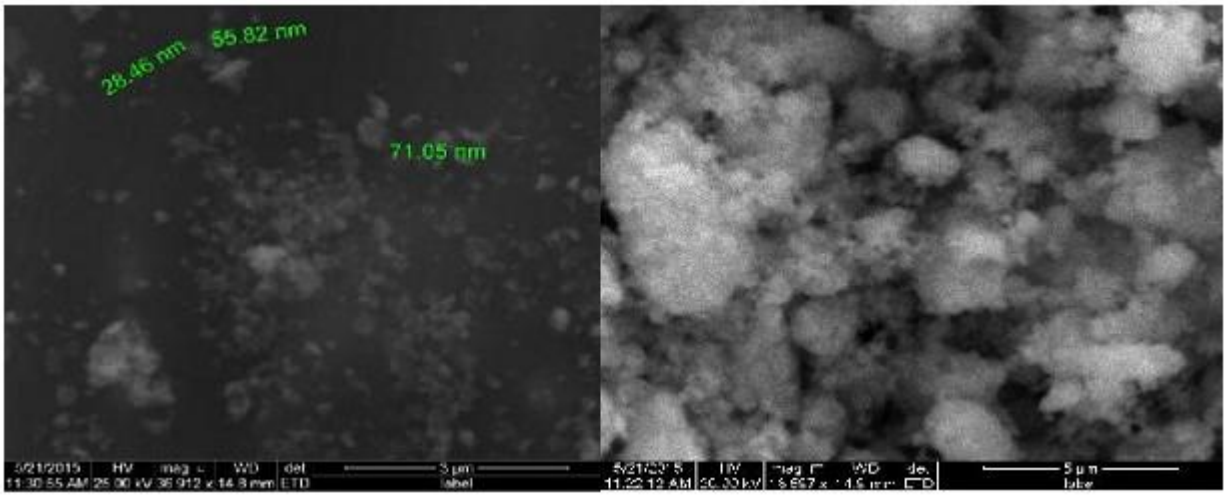

Figure (2):SEM picture and size distributions of ZnO NPs

Effect of ZnO NPs on AST activity:

The results in Table (4) demonstrated the activator effect of ZnO NPs on salivary AST activity, Figure (3) shows the median values of enzyme activity for the studied groups.The activity of AST (mean ± SD) in presence of nanoparticles of zinc oxide (2.382 ±0.177) was higher than its activity in patient's saliva without NPs (1.210 ±0.702), also the results were revealed that the enzyme activity in both patient groups (with and without ZnO NPs) was higher than its activity in control group (0.420 ±0.247). Highly significant differences were showed among the studied groups (p=0.000). These results were illustrated in figure (3) which was showed the effect of ZnO NPs on salivary ALP clearly.

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

Groups	Descriptive statistics						
	N	Median	Mean	S.D.	Min.	Max.	p-value
AST activity in control	20	0.355	0.420	0.247	0.16	1.1	0.000 (HS)
AST activity without NPs	60	0.98	1.210	0.702	0.16	2.99	
AST activity with NPs	60	2.05	2.382	1.770	0.56	9.21	

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

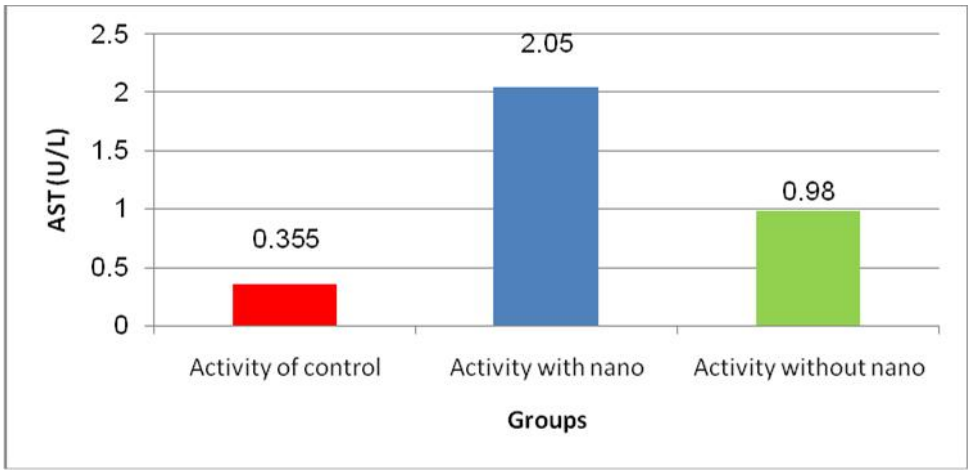

Figure (3): Median of AST activity in patients with and without ZnO NPs and control group.

Table (5) shows the results that obtained from an additional analysis using Mann – Whitney U test; data were computed to determine the significant differences between each two groups of the experimental groups in this study. The results of this analysis were revealed a highly significant differences (p=0.000) between groups of control versus patients with nano, control versus patients without nano and patients with nano versus patients without nano

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

Groups Statistical parameters	Control vs. Patients with nano	Control vs. Patients without nano	Patients with nano vs. without nano
Mann-Whitney U	33	145.5	959
p-value	0.000 (HS)	0.000 (HS)	0.000 (HS)

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

Figure (4) was showed the effect of ZnO NPs (µg/ml) on the activity of salivary AST (U/L) in a constant total volume of reaction mixture (600 µl). The greater activation effect by ZnO NPs on this enzyme activity was found to be at concentration of 0.33 µg/ml in total volume of the reaction mixture.

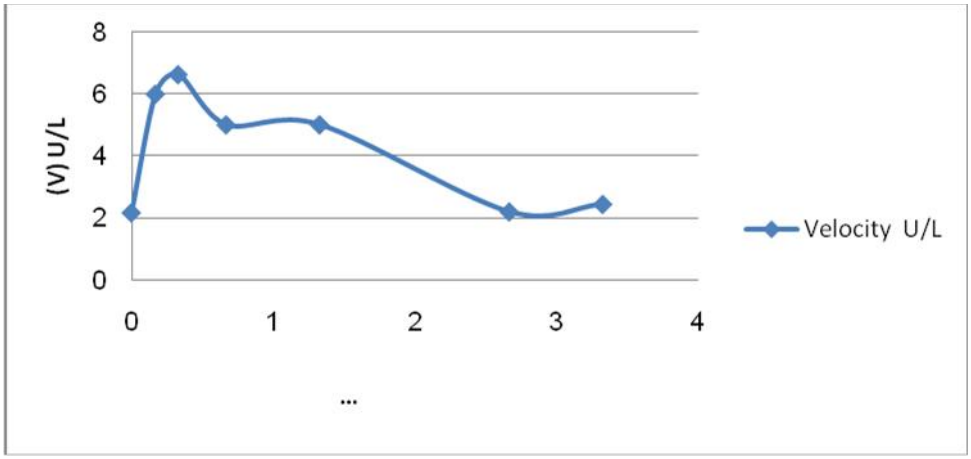

Figure (4): Salivary AST activity in presence of different concentration of ZnO NPs.

The greater activation percentage of AST activity by ZnO NPs was found to be of 65.25٪ at concentration of 0.33 µg/ml, as a more effective NPs concentration among others.

The present work considers the first study that demonstrates the effects of ZnO NPs on salivary AST activity in chronic periodontitis patients. Our results indicated that there is activation effect of these NPs on enzyme activity. Pandurangan and Kim showed that ALT, AST, ALP and LDH enzymes activities were significantly increased in a dose-dependent manner by ZnO NPs and significantly produced cytotoxicity in C2C12 cells [30].

Moderate concentration of zinc oxide nanoparticles leads to an increase in serum LDH activity comparing with control group in male mice [31]. In previous study, the activation effects of gold and silver nanoparticles on choline esterase and monoamine oxidase activities were demonstrated [32]. AL-Rubaee [33] showed that the effect of gold nanoparticles on salivary LDH activity increased with different concentration of the nanoparticles. The effects of gold and silver nanoparticles were studied on the activities of serum AST and ALT enzymes, inhibitor effects were demonstrated, and these effects increased with the increasing of nanoparticles concentrations [34].

ZnO NPs have been widely used in production of food and in medicine [35]. Zinc concentration are associated with many metabolic and antioxidant enzymes [36]. High level of zinc is important for cells and zinc is a component of several enzymes and transcription factors [37].

Conclusion

the results show increasing of salivary AST activity in presence of ZnO NPs, this activation effect may be interpreted on the basis of the conformational changes that can be occurring on the protein structure after interaction with ZnO NPs.

References

1. SalataOV. (2004). Applications of nanoparticles inbiology and medicine. J. Nanobiotechnology. doi:10. 1186/1477-3155-2-3.
2. Leka´a M. Ibrahim, Kadhim K. Ghudhaib, Eaman A. Al-Rubaee,Zainab A. Salman. (2016). Estimation of ZnO nanoparticles effect on salivary ALP activity in chronic periodontitis Patients: *in vitro* study. Int. J. Adv. Res. Biol. Sci. 3(4): 152-159
3. Kadhim K. Ghudhaib, Leka´aM. Ibrahim, Eaman A. Al-Rubaee,Zainab A. Salman. (2016). Effect of ZnO nanoparticles on salivary Peroxidase activity in chronic periodontitis patients (in vitro study). IOSR-J Appl. Chem. Accepted.
4. Eaman A.S.AL-Rubaee, Abdulrahman K Ali,Amenah Ali Salman, Zainab A.Salman. (2015).Inhibition effect of noble metals nanoparticles on acid Phosphate activity in sera of healthy subject. Eng. &Tech. J, B. 33(1): 1-11.
5. Eaman A. S. AL-Rubaee, Suha T. Abd, Nadia M.Kadim. (2015). The effect of titanium oxide nanoparticles on salivary alkaline phosphates activity. Inter J Res. Develop. Pharm. Life Sci. 4(6) pp 1813-1819
6. Eaman A. S. AL-Rubaee.(2014). Kinetic study of the effect of gold and silver nanoparticles on salivary LDH activity. Global J Sci. Res.2(4): 111-118
7. Zarrindokht, E.K., Pegah, C. (2011). Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. African Journal of Microbiology Research, 5(12): 1368-1373.
8. Amna, A. S. (2012). Antibacterial activity of ZnO nanoparticle on some gram-positive and gramnegative bacteria. Iraqi J Phys., 10 (18): 5-10.
9. Zhao, J., Castranova, V. (2011). Toxicology of nanomaterials used in nanomedicine. J Toxicol. Environ. Health B, 14: 593–632.
10. American Academy of Periodontology. (2004). Drug-associated gingival enlargement. Informational paper. J Periodontal. 75:1424-1431
11. Nagarajan, S., Kuppusamy, K. A. (2013). Extracellular synthesis of zinc oxide nanoparticle using seaweeds of Gulf of Mannar India. Journal of Nanobio-technology.11:39
12. Numabe Y, Hisano A, Kamoi K, Yoshie, Ito K, Kurihasa H. (2004). Analysis of saliva for periodontal diagnosis and monitoring. Periodontol 2000. 40:115–9.
13. Ozmeric N. (2004). Advances in periodontal disease markers. Clin Chim Acta.343:1–16
14. 14-Kudava P, Saini N, Kudva H, Saini V. (2014). To estimate salivary aspartate aminotransferase levels in chronic gingivitis and chronic periodontitis patients prior to and following non-surgical periodontal therapy: A clinico-biochemical study. J Indian Soc Periodontal. 18(1):53-8.
15. Lang NP, Bartold PM ,Cullinam M, et al. International classification workshop. Consensus report: chronic periodontitis. Annals of Periodontology, 1999; 4:53.
16. Carranza. Carranza´s Clinical periodontology, 11 th edition, 2012.
17. Retman , S. , Frankel , S.(1957). Chemhouse. J. Clin. Path.28:56 .
18. Cesco, Rde, T., Ito, I.Y., and dealbuquerque, R.F.(2003). Levels of Aspartate aminotransferase (AST) in saliva of patients with different periodontal conditions. J Clin Periodontol .30(8): 752- 5.
19. Todorovic, T., Dozic, I., Barrero, M. V. , Ljuskovic, B., Pejovic, J., Marjanovic, M., and Knezevic, M.(2006). Salivary enzymes and periodontal disease. Med Oral Patol Oral Cir Bucal. 11:E115-9.
20. Totan ,A., Greabu, M., Totan, C., and Spinu, T.(2006). Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase: possible markers in periodontal diseases. Clin Chem Lab Med .44(5):612-5.
21. Magnusson, I., Persson, R.G., , Page, R.C., DeRouen, T.A., Crawford, J.M., Cohen, R.L., Chambers, D.A., Alves, M.E., and Clark, W.B.(1996). A multicenter clinical trait of a new chairside test in distinguishing between diseased and healthy periodontal sites. Association between site type and test outcome before and after therapy. J. Periodontol .67(6):589-96.
22. Wong, M.Y., Lu ,C.L., Liu ,C.M., Hou, L.T., and Chang, W.K.(1999). Relationship of the subgingival microbiota to a chairside test for aspartate aminotransferase in gingival crevicular fluid. J. Periodontol 70(1):57-62.
23. Luke, R. , Khan, S. N. , Iqbal, P. S. , Soman, R.R., Chakkarayan, J., and Krishnan, V.(2015). Estimation of Specific Salivary Enzymatic Biomarkers in Individuals with Gingivitis and

Chronic Periodontitis : A clinical and Biochemical Study. J. of International Oral Health. 7(9): 54-57.
24. Popovic, Z., Brkic, Z. , Radicevic, B. A.,Milicic, B., Dozic, I .(2013). Analysis of Intracellular Enzymes in saliva of Patients with Aggressive Periodontitis. Serbian Dental Journal. 60 (3):155-158.
25. Mohammad, A. N.(2011). Salivary enzymes as markers of chronic periodontitis among smokers and non smokers .J Bagh College Dentistry .Vol. 23(3): 83- 87.
26. Wahab, G. A., Ahmed, M.A. (2015). Assessment of some salivary enzymes levels in type 2 diabetic patients with chronic periodontitis (clinical and biochemical study). J Bagh College Dentistry .Vol. 27(1):138-143
27. Kaufman, E., Lamster, I.B.(2000). Analysis of Saliva for Periodontal Diagnosis. J Clin Periodontol . 27: 453-465
28. Ozmeric N.(2004). Advances in periodontal disease markers . Clin Chim Acta 343- (1-2):1-16. [Pub Med: 15115674] .
29. Numabe, Y., Hisano, A., Kamoi, K., Yoshie, H., Ito, K.,and Kurihara, H. (2004). Analy¬sis of Saliva for Periodontal Diagnosis and Monitoring. J Periodontol . 40:115-9.
30. Muthuraman, P. Doo, H.K. (2015). ZnO nanoparticles augment ALT, AST, ALP and LDH expressions in C2C12 cells. . Saudi Journal of Biological Sciences.22.679-684.
31. Negahdary, M., Ajdary, M. (2014) .The toxicity of gold, silver, and zinc oxide nanoparticles on LDH enzyme in male mice .Annual Research & Review in Biology 4(8):1346-1352.
32. Abbas, S. A.(2011). The effect of gold and silver nanoparticles on choline esterase and monoamine oxidase enzymes activities. INT. J. Chemistry., 3(4): 61- 68.
33. AL- Rubaee E.A.(2014).Kinetic study of the effect of gold and silver Nanoparticles on salivary LDH activity.GJSR Journal. 2(4), pp.111-118.
34. Abbas, S. A. R. Abdullah, A. H. Abdul, H. S. and Ali, S. A. K.(2011). The Effectes Of Gold and Silver Nanoparticles on Transamenase Activities. J. Chem. Res. (4):1-11.
35. Van Tassel, K.A., Goldman, R., (2011). The growing consumer
36. Kumar, A., Pandey, A.K., Singh, S.S., Shanker, R., and Dhawan, A.,(2011).Engineered ZnO and TiO2 nanoparticles induce oxidative stressand DNA damage leading to reduced viability of Escherichia coli.Free Radic . J.Toxicol. Environ. Health A 73, 166–174.
37. Asuri, P., Bale, S. S., Pangule, R. C., Shah, D. A., Kane,R. S. and Dordick, J. S.(2007). Structure, Function, and Stability of Enzymes Covalently Attached to Single-Walled Carbon Nanotubes. Langmuir, 23(24): 12318-12321.

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