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KINETIC STUDY OF THE EFFECT OF GOLD AND SILVER NANOPARTICLES ON SALIVARY LDH ACTIVITY

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ABSTRACT
Noble metals nanoparticles have been prepared by a facile and clean synthesis method by laser ablation in water. The structural and optical properties of the Ag and Au nanoparticles (NPs) have been investigated. The produced nanoparticles have an average particle size of about 30 nm. The effect of gold and silver NPs was studied on the activity of lactate dehydrogenase (LDH) in saliva of healthy subjects. The results correlated with the observation that gold and silver nanoparticles have different effect on salivary LDH activity, and this effect increased with increasing the concentration of the nanoparticles. While gold nanoparticles demonstrated activator effect on salivary LDH activity, silver nanoparticles have inhibition effect on it.

KEYWORDS: Au and Ag nanoparticles, LDH activity, Saliva, Kinetic study.

INTRODUCTION

In the recent past nanomaterials emerged and took their place in lots of field, such as electronics, optics, cosmetics, and food. Nanomaterials were also used in medicine and diagnostics. This dramatic increase of industrial production and use of nanomaterials has led to investigate their potential health effects. Indeed the nanotoxicology is a field well established. Studies of the effects of nanoparticles (NPs) from different industry branches on cells and pathways are emerging, and most of the biological effects of NPs seem due to their interactions with proteins[1-5]. Enzymes are biologic polymers that catalyze the chemical reactions that make life possible. With the exception of catalytic RNA molecules, or ribozymes, enzymes are proteins. The ability to assay the activity of specific enzymes in blood, other tissue fluids, or cell extracts aids in the diagnosis and prognosis of disease[6]. Lactate dehydrogenase (LDH) is an enzyme that catalyzes the interconversion of lactic and pyruvic acids. It is a hydrogen-transfer enzyme that uses the coenzyme NAD+ according to the following equation:

$$\underset{\text{Lactate}}{CH_3CHOHCOOH} + NAD^+ \leftrightarrow \underset{\text{Pyruvate}}{CH_3COCOOH} + NADH + H^+$$

LDH is widely distributed in the body. High activities are found in the heart, liver, skeletal muscle, kidney, and erythrocytes; lesser amounts are found in the lung, smooth muscle and brain. It is elevated in a variety of disorders. Increased levels are found in cardiac, hepatic, skeletal muscle, and renal diseases, as well as in several hematologic and neoplastic disorders[7,8]. Saliva has been used as a diagnostic fluid in medicine and dentistry. It is easy to collect using non-invasive methods. The intracellular enzymes present in saliva have been studied as markers of periodontal disease. Host responses to periodontal disease include the production of different enzymes that are released by stromal, epithelial or inflammatory cells. There are important enzymes associated with cell injury and cell death like: aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acidic phosphatase (ALP, ACP), gama glutamyl transferase (GGT). Changes in enzymatic activity reflect metabolic changes in the gingiva and periodontium in inflammation[9-11]. Enzyme kinetics is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme –catalyzed reactions and the systematic study of factors that affect these rates. A complete, balanced set of enzyme activities is of fundamental importance for maintaining homeostasis. So, understanding of enzyme kinetics is important to understanding how physiologic stresses such as anoxia, metabolic acidosis or alkalosis, toxins, and pharmacologic agents affect the balance[12] . There are many articles show the scientific data on the deleterious effects of NPs on key mediators of biological functions such as enzymes [13-15]. There is no study about the effect of gold and silver NPs on LDH activity in saliva; therefore, this study was conducted to investigate the effect of these particles on LDH activity in human saliva in vitro.

MATERIALS & METHODS

Nanoparticles

Gold and silver nanoparticules have been obtained from school of applied Science, University of Technology, Iraq. The laser used in this experiment was Nd: YAG laser (type HUAFEI). The laser beam was focused by a lens onto a pure (99.999) metals target, which was submerged in de-ionized water inside of plastic vessel. Absorbance spectra of NPs solution were measured by UV-VIS double

beam spectrophotometers, CECIL C. 7200 (France) and SHIMADZU. All spectra were measured at room-temperature in a quartz cell with 1 cortical path. Atomic absorption spectroscopy AAS measurement was carried out for the prepared samples using AAS spectrometer model GBS 933, Australia. Structure and nano size measurement of nanoparticles samples were identified by the transmission electron microscope TEM type CM10 pw6020, Philips-Germany (Electronic Microscope Centre-Collage of Medicine/ Al-Nahrien University). The test samples were prepared by placing a drop of suspension of interest on a copper mesh coated with an amorphous carbon film. The drop was dried with an infrared lamp (Philips, 100 W) until all the solvent had evaporated. This process was repeated three to four times.

Salivary Lactate Dehydrogenase assay

The salivary lactate dehydrogenase activity was spectrophotometrically determined according to the recommendation of the French Society of Clinical Biology (FSCB) with specific reagents (Biomaghreb Kit). The reaction mixture contained a substrate 1.6 mmol/L pyruvate, 0.2 mmol/L reduced nicotinamide adenine dinucleotide (NADH), 80 mmol/L Tris buffer (pH 7.2 at 30 °C) and 200 mmol/L NaCl, in a total volume of 1.0 ml. In the presence of lactate dehydrogenase (200 µL of saliva), pyruvate is reduced to L-lactate with the simultaneous oxidation of NADH. The rate of decreased in absorbance at 340nm -representing the NADH consumed- is directly proportionate to the LDH activity in the sample. For the 1cm path length used, a value of 6.22 was considered as the NADH millimolar absorptivity. Results were first converted into enzyme activity units (1 unit= 1µmol of NAD+ released per minute) and finally expressed as total LDH activity (units/L) per sample[10].

Collection of Saliva

Saliva collection was performed 2-3 hours after the volunteer usual breakfast time and after thoroughly rinsing the mouth with water .Saliva was collected by standard spitting method using chewing then saliva collected in a plane tube, centrifuged 10 minute at 1500 xg, and the supernatant liquid was used for analysis immediately.

Effect of gold and silver nanoparticles on salivary LDH activity:

From a stock (15µg/ml) concentration of silver NPs and a stock (20µg/ml) concentration of gold NPs, the following concentrations (1, 2, 4, 7.5) µg/ml of silver NPs and (1.3, 2.7, 5.4, 10.0) µg/ml of gold NPs were prepared as a final concentration on the total reaction mixture by diluting with deionized water. The percentage effect on activity was calculated by comparing the activity with and without the gold or silver NPs and under the same conditions according to the following equations:

% inhibition = 100-100x (Activity in the presence of nanoparticles/ Activity without the nanoparticles)

% activation = (Activity in the presence of nanoparticles / Activity without the nanoparticles) x100 – 100

A constant concentration of Au NPs (2.5 µg/ml) and Ag NPs (2.5µg/ml) were used with different substrates concentrations (0.1, 0.3, 0.5, 0.8, 1.1) mmole/L as a final concentrations in the reaction mixture. The enzyme activities were determined with and without the NPs by using Lineweaver-Burk equation. Apparent V_{max}, Apparent K_m and type of inhibition were evaluated[16].

Statistical analysis

Statistical analysis was performed using SAS (version 9.1 2010) and Microsoft Office Excel (Microsoft Office Excel for windows; 2003). Data were analyzed by using Two Way Analysis of Variance (ANOVA). Student T-test was used to assess significant difference among means at level ($P < 0.05$).

RESULTS & DISCUSSION

Figure (1) shows the colloidal nanoparticles produced by laser ablation of pure metal plate of gold and silver immersed in pure water exposed by 100 laser pulses, with laser flounce (F= 40 J/cm^2, the laser spot size is 1.27 mm), at laser wavelength of 1064.

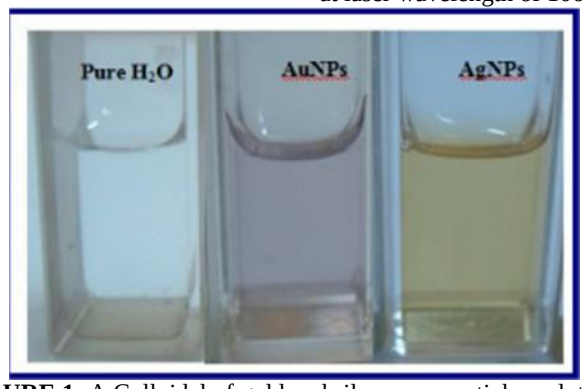

FIGURE 1: A Colloidal of gold and silver nanoparticles solutions

A visible coloration of the solution after several pulses during the experiment was observed. The color of solutions is faint pink for gold nanoparticles and yellow color for silver nanoparticles. The color of metal nanoparticle is resulted from the coherent oscillation of the conduction band electrons for metallic nanoparticles can be induced by the interacting electromagnetic field, which is named as surface plasmon extinction[17]. Fig. 2 and 3 shows the UV-VIS absorption spectra that indicated the characteristic absorbance feature of silver and gold nanoparticles, respectively. This was carried out by pulsed laser ablation of a metal plate in water. A focused Nd-YAG laser operated at 1 Hz with a wavelength of 1064 nm was vertically irradiated onto a metal plate placed in the aqueous solution. The ablating energy of 600 mJ was employed to ablate a target. The products formed in the

ambient liquid were transparent just after ablation, and then changed to contaminated ones after more application of NPs. Fig. 2 illustrate absorption spectra of gold nanoparticles, the surface plasmon related peak could be clearly distinguished. This peak was around 530 nm, which was consistent with the presence of small 3–30 nm Au nanoparticles in the solution[18].

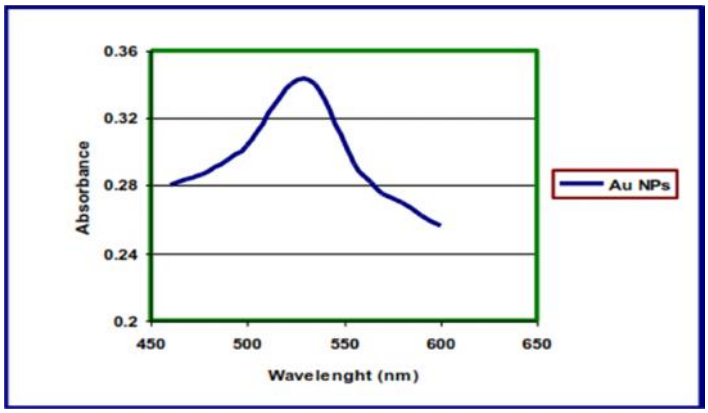

FIGURE 2: Absorbance spectra of the gold (Au) nanoparticles, obtained by laser ablation of metal plates immersed in pure water. The laser shots are 90 pulses at laser energy of 600 mJ and λ=1064 nm

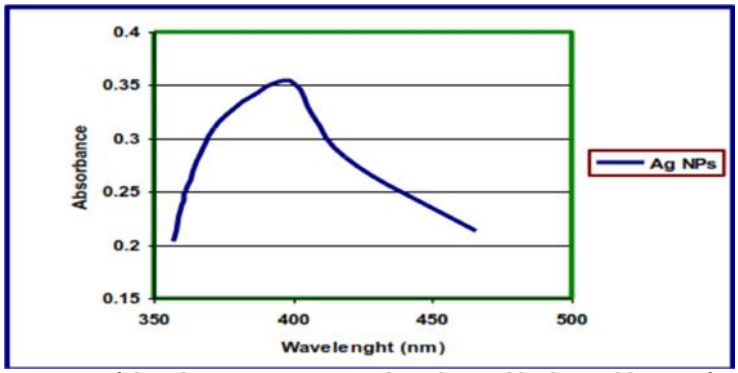

FIGURE 3: Absorbance spectra of the silver (Ag) nanoparticles, obtained by laser ablation of metal plates immersed in pure water. The laser shots are 90 pulses at laser energy of 600 mJ and λ=1064 nm

Fig. 3 shows UV–VIS absorption spectra of Ag NPs. All the spectra exhibit a characteristic peak around 400 nm, indicating the formation of Ag Nano colloids[19]. Figure 4(A and B) shows TEM pictures and size distributions of gold nanoparticles, produced by laser ablation of metal plates immersed in DDDW; the laser wavelength is 1064 nm. The nanoparticles thus produced were calculated to have the average diameters of 30 nm at the laser energies 600mJ.

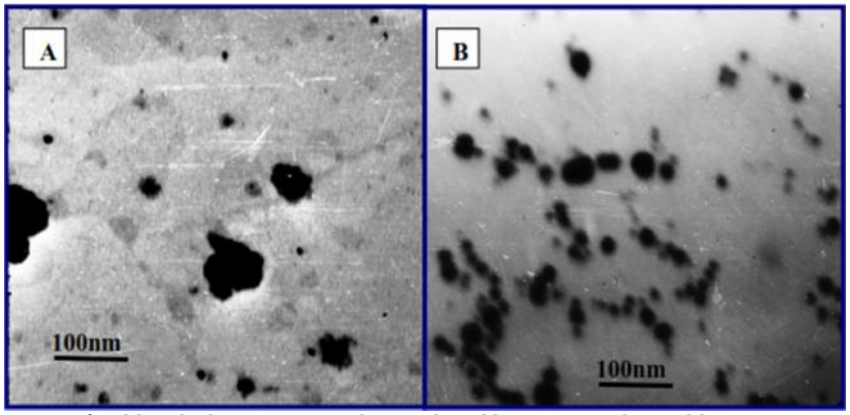

FIGURE 4: TEM images of gold and silver nanoparticles produced by 1064-nm laser ablation (E=600 mJ/pulse) of metals plate immersed in 1ml of pure water.

The average particles sizes increase and the size distribution broadens with an increase of applied laser energy. The origin of the surface morphology of the irregularly shaped particles in case of high energy can be

explained by absorption by defects and thermally induced pressure pulses which cause cracking[20].This fragmentation mechanism explained the variation in size distribution. Therefore the population of particles smaller than 10 nm increased markedly in solution when laser energy decrease, compared to higher laser energy. However the density of the ablated species can be changed by adjusting the laser energy.

TABLE 1: The kinetic properties of salivary LDH activity with and without Ag nanoparticles

	Vmax (U/L)	Km (mmol/L)
Without Ag-NPs	68.45	0.565
With Ag-NPs	29.33	0.193

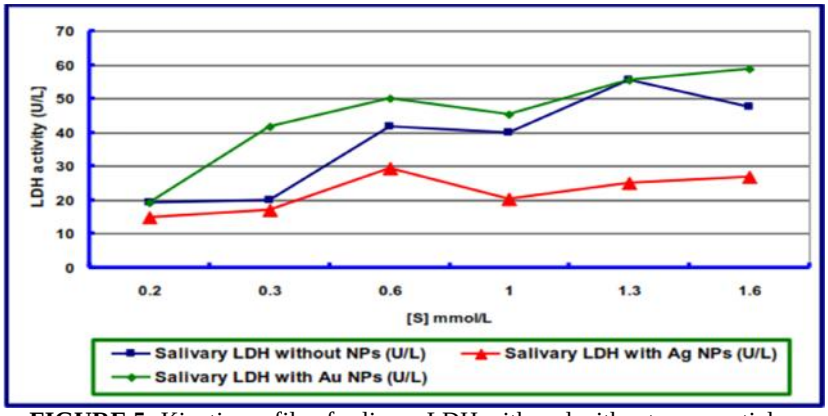

FIGURE 5: Kinetic profile of salivary LDH with and without nanoparticles

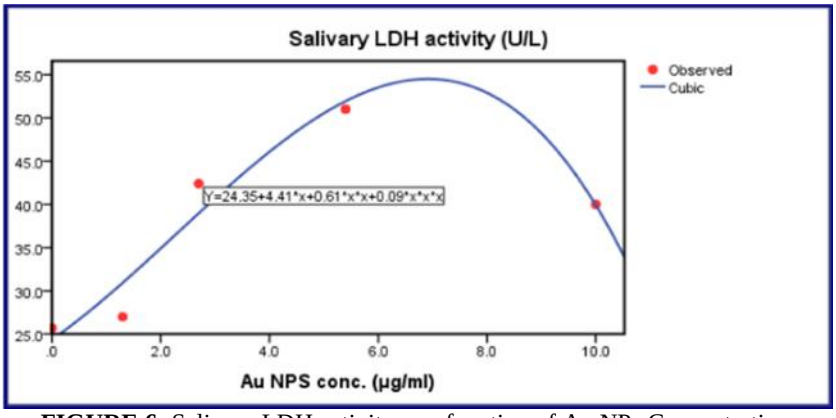

FIGURE 6: Salivary LDH activity as a function of Au NPs Concentration

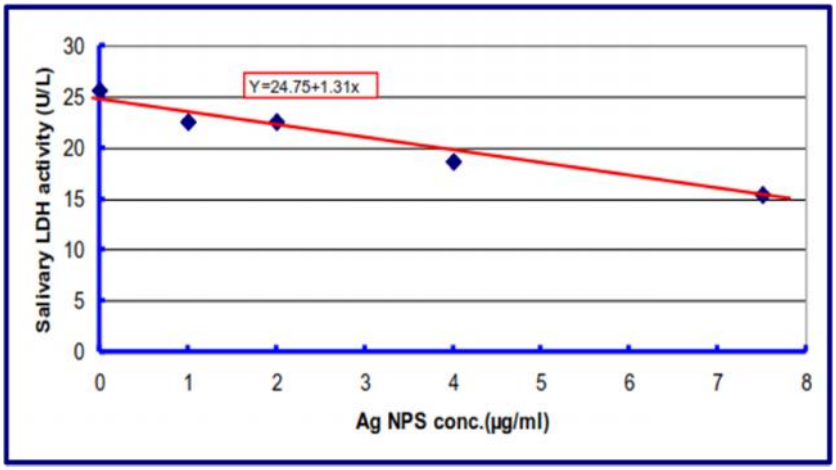

FIGURE 7: Salivary LDH activity as a function of Ag NPs concentration

The kinetic biochemical tests revealed that NPs of Au and Ag caused deferent effect on salivary LDH activity as shown in figure(5), the relationships between NPs of Au and Ag concentrations versus the activity of enzyme are

shown in figures (6) and (7). These results observed that any increase in Ag nanoparticles concentration caused increasing in percentage of inhibition of enzyme activity. The greater inhibition of Ag NPs on enzyme activity was 40.1% at concentration (7.5) µg/ml (figure 8).While there was an activation effect of Au NPs on enzyme activity, any increase in concentration caused increasing in percentage of activation of enzyme activity. The greater activation of Au NPs on salivary LDH activity was 98.4% at concentration (5.4) µg/ml (figure 9). Table (1) and figure(10) showed that the kinetic parameters K_{mapp}, V_{maxapp} and type of enzyme inhibition using Lineweaver-Burk equation for Ag NPs on salivary LDH activity. The V_{max} and K_m without Ag nanoparticles were (68.45) U/L, and (0.565) mmole/L respectively. A liquate (2.5)µg/ml of Ag NPs was uncompetitive inhibition for enzyme activity. The V_{maxapp} and K_{mapp} were (29.33) U/L, and (0.193) mmole/L respectively. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme [16, 21, 22].

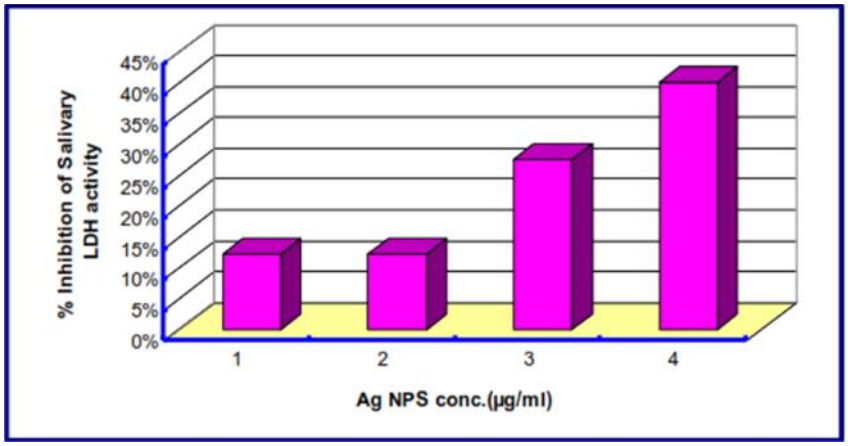

FIGURE 8: Percentage inhibition of salivary LDH activity as a function of Ag NPs

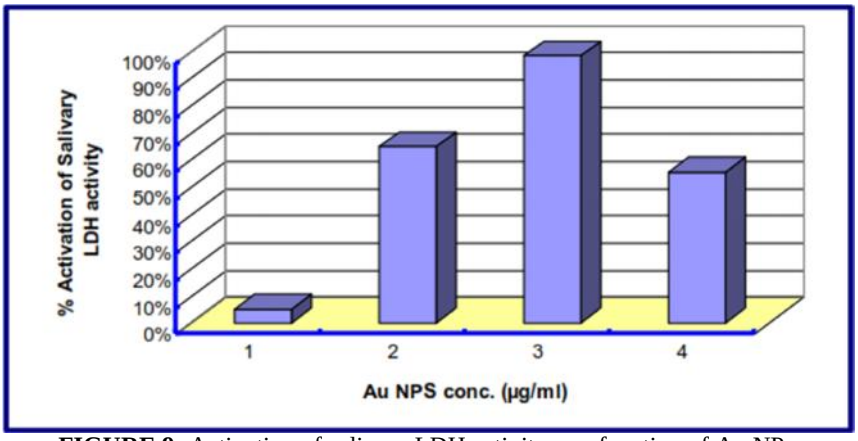

FIGURE 9: Activation of salivary LDH activity as a function of Au NPs

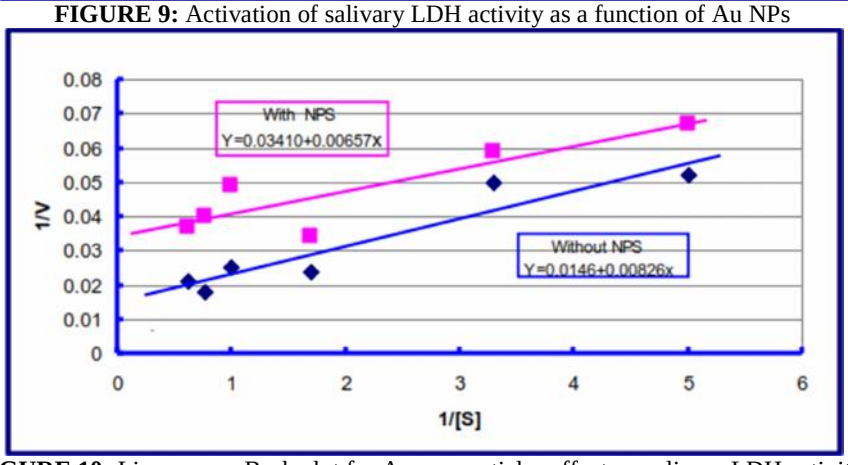

FIGURE 10: Lineweaver-Burk plot for Ag nanopaticles effect on salivary LDH activity.

In a recent study, Negahdary and Ajdary [23] reported that a moderate concentration in each of gold, silver, and zinc oxide nanoparticles leads to an increase in serum LDH activity as compared with control group in male mice. In another study of the effects of silver nanopartticles on LDH activity and histological changes of heart tissue in

Wister rats, results showed that different concentrations of AgNPs have no significant effect on the serum LDH activity[24]. While, in a study on kinetic and physicochemical properties of protein or enzymatic products in the presence of silver nanoparticles, the results showed that nanosilver could significantly decrease LDH activity and by florescence spectral assays, the silver nanoparticle was determined to be directly bound to LDH and induced the protein unfolding[25]. Heavy metals are toxic and react with proteins, therefore they bind protein molecules, heavy metals strongly interact with thiol groups of vital enzyme and inactivate them[26]. In addition, it is believed that Ag bind to functional groups of proteins, resulting in protein deactivation and denaturation [27, 28]. This study is the first that demonstrates the effects of gold and silver nanoparticles colloids on the salivary LDH activity. Gold nanoparticles has attracted a continuous interest due to their unusual properties in DNA hybridization[29, 30], and biocatalysts[31]. In recent years, incorporation of nano-silver into medical products has been of great interest. Properties of nano-structured silver can be controlled and tailored in a predictable manner and impart them with biological properties and functionalities that can bring new and unique capabilities to a variety of medical applications ranging from implant technology and drug delivery, to diagnostics and imaging[32]. Several mechanisms have been postulated for the antimicrobial property of Ag-nanoparticles [26, 27]. Only a few studies are conducted on the crucial metabolism enzyme and enzyme dysfunctions, which are related to various pathologies [13,14]. Therefore, it was useful to know the effect of gold and silver nanoparticles on activity of lactate dehydrogenase in saliva. More studies are need to explain the activation effect of gold nanoparticales on salivary LDH activity. The nanoparticles induced protein modifications are promising fields for future research. Proper understanding of such phenomenon is further emphasized by the fact that these materials are utilized for diagnostic and therapeutic purposes.

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