Original research

Cytotoxicity effect of lectin coated nanoparticles on Balb/c mice cells

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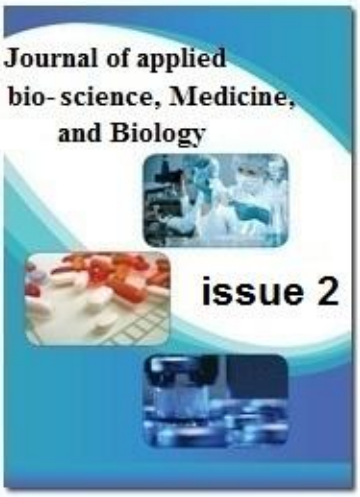

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Abstract:

Different types of nanoparticles are using in medical and technological sciences. Unfortunately , the most nanoparticle have cytotoxic effects that is dependent on their chemical composition, size, surface ratio and surface modification or coating also the use of electrical, magnetical or electromagnetic waves change nanoparticle cytotoxicity. In this research, cytotoxicity of Titanium Oxide nanoparticles(TiO2 NPs), Zinc oxide nanoparticles(ZnO NPs), Magnesium oxide nanoparticles(MgO NPs), Silver nanoparticles(Ag NPs) , Gold nanoparticles(Au NPs) and lectin coated of these nanoparticles with Ultra Violet (UV) and Infra Red (IR) irradiation on different cells(skin, macrophage, heart, liver and kidney) of Balb/c mice were measured by 5-diphenyl-tetrazolium bromide (MTT) ,lactate dehydrogenase (LDH) enzyme release , quantity of cell metabolic function and cell ATP level.This research showed that there was a direct relationship between nanoparticle concentrations and related cell cytotoxicity and the use of UV and IR exposure lead to increasing of nanoparticle cytotoxic effect and lectin coating lead to decreasing of nanoparticle cytotoxic effect. Also, Ag and Au nanoparticles are more cytotoxic than metal oxide nanoparticles.

Keywords: Cytotoxicity; Nanoparticles; irradiation; Ultra Violet; Infra Red

Introduction:

Different types of nanoparticles are prospering in medical and technological sciences and can be used in nanobiosensors, DNA and protein assay, tissue engineering and drug-delivery systems(1-5). The demeanour of nanoparticles is comparatively different depend on size. There is a relationship between a decreasing in size and an increasing in cytoxicity. Because it has larger surface area(6). Some nanoparticles can show toxic properties that can lead to cell damage including inflammation and other toxic consequences(7). These damages may lead to asthma and atherosclerotic heart diseases for air-born nanoparticles(8). The study on toxicity of nanoparticles informs that nanoparticles can introduce the human body and turn toxic for tissues and organs. As soon as nanoparticles move in to the body, these can displace in the blood stream throughout the body and arrive at the liver, kidney or others organs. It can get deeper into the lungs and may go across the blood brain barrier(BBB) also Skin contact may happen with nanoparticles handling(9-12). It has been shown that nanomaterials have highly surface area which are able to carcinogenic and mutagens activity (13-14). Nanoparticles intervene with proteins, DNA and cell membranes function and some reports showed that some nanoparticles results to DNA damage , caspase activation and lipid peroxidation induced by condensation of chromatin, formation of micronuclei and lead to cell apoptosis or necrosis(15-17).Therefore, knowing nanoparticles interaction with different cells is important and must be studied all aspect of hazard and health crisis of nanomaterials.

The influence of nanoparticle interactions is dependent on their chemical composition, size, surface/volume ratio and surface modification or surface coating also the use of electrical, magnetical or electromagnetic waves enhance nanoparticle cytotoxicity(20-

22,24). Nanoparticles are extremely reactive with oxygen molecule after irradiation to UV light and generate hydroxyl radical (OH-) and superoxide anion ($O2^-$) witch gives nanoparticles momentous cytotoxic like TiO2 and ZnO and Au nanoparticles. Reactive oxygen species (ROS) that have the capability to devastate cellsAuNPs with smaller size showed a bigger yield of ROS production. An inverse ratio of ROS generation size proposes a catalytic function of AuNPs surface for increased ROS generation (20-22).Some nanoparticles like AuNPs with near-infrared (NIR) can induce photothermal effect(23-24).These nanoparticles are exposed to a NIR laser light and the nanoparticles absorb the energy and alter it to heat that increases the temperature and take cells away by breaking the membrane. The usage of photothermal toxicity by non-invasive NIR energy is limited because of no deeper entrance. Hyperthermia by AuNPs and NIR, is presently being researched in clinical trials (23-24).The chemical composition of nanoparticle surface is important in cytotoxicity properties. Serum protein adsorption of nanoparticle surface lead to uptaking of nanoparticles via receptor-mediated endocytosis and can alter nanoparticle Cytotoxicity (25-28). If proteins have no receptor on cells, nanoparticles may not enter to cell by receptor-mediated endocytosis and the Cytotoxicity of nanoparticles may changed. This field is new and have little information and articles about it(28).

In this research, cytotoxicity of TiO2 NPs, ZnO NPs, MgO NPs, Ag NPs, Au NPs and pea nut agglutinin(PNA) lectin coated of these nanoparticles with UV and IR irradiation on different cells of Balb/c mice were measured by MTT assay , LDH release, quantity of cell metabolic function and cell ATP level.

Materials and methods:

-Preparation of nanoparticles:

TiO2 NPs, ZnO NPs, MgO NPs, Ag NPs and Au NPs were obtained from Lolitech company, Germany and used without further purification. The wide range of concentration from all-NPs (2000, 200, 20, 2, 0.2 and 0.02 mg/L) was prepared in phosphate buffered saline(PBS) and used in study.

Preparation of lectin coated nanoparticles:

For coating of nanoparticles**,** One ml of PNA lectin(Sigma, German) at concentration 2mg/ml was added to one ml of each nanoparticles suspension at concentration 2000 mg/L and mixtures were shaked and incubated 1 hour at 37 ^{0}c. After incubation the mixture was centrifuged at 10000 rpm and the supernatant collected. Finally pellets were resuspended in one ml of PBS and other concentration(200, 20, 2, 0.2, 0.02 mg/L) were prepared from 2000 mg/L of lectin coated nanoparticles suspension. A UV-visible spectrophotometer (ELICO, India) served to determine the protein concentration at 280nm also Fourier transform infrared spectroscopy (FTIR)(ELICO, India) was used for lectin adsorption confirmation.

Characterization of nanoparticles:

The structure, size and composition of all nanoparticles and coated nanoparticles were characterized by FTIR, Scanning Electron Microscopy (SEM)(Hitachi S-2400) and Dynamic light scattering(DLS)(Malvern Instruments,Italy).

For SEM, A drop of the sample solution was dried on a copper holder and coated with a thin layer of gold by sputtering prior to microscopic examination then samples were

examined using SEM with BSE detector (Back Scaterred Electrons) and EDS (Energy Dispersive X-ray Spectroscopy).

DLS analysis were performed by Malvern Zetasizer Nano ZS apparatus at 25 ºC and started 2 min after the cuvette was placed in the DLS equipment to allow the temperature to balance. Analysis were carried out 1 h after the preparation of the suspensions. Nanoparticle suspention was made in deionized water(DW)

The FTIR experiment were carried out with a spectrophotometer in the range of 4000-400 cm–1, using the KBr technique, which involves combining exhaustively the material to be tested with KBr before shap a pellet at high pressure(29).

-Animals:

Balb/c female mice (18-20 g) were purchased from istitue pastur ,Iran. Procedures were conducted in according to institutional guidelines and international laws and policies (EEC Council Directive 86/609, OJ L 538,1, December 12, 1987; National Institutes of Health [NIH] Guide for the Care and Use of Laboratory Animals, NIH publication No. 85-23, 1985).

-Preparation of mice cells:

Different tissues (skin, heart, liver, kidney) and peritoneal macrophage cells were prepared aseptically and rinsed in Hanks' balanced salt solution (GIBCO). Tissues were cutted into 2×2-mm squares, and digested in 20 mL of trpsin enzyme at concentration 1 mg/mL (Sigma,German) at 37°C for 30 minutes with mild agitation. The cellular digest was centrifuged at 400*g* for 15 minutes, and washed twice in 10% FCS–RPMI1649; the cell pellet was resuspended in 10 mL of 10% FCS–RPMI1649 until reached one million per ml.

-MTT assay or cell mitochondrial function:

Different mice cells added to different concentration of naked nanoparticles and lectin coated nanoparticles and incubated 24 hour at 37 ^{0}c with UV irradiation(200-300nm,8watt, Ultra-Lum Inc.,USA) and another with IR irradiation (800-1400nm,8w, TUNGSRAM-SCHREDER , HUNGARY) and without irradiation(dark condition) and were then washed three times with HBSS and and then 100 µL of RPMI1649 media with 1% L-glutamine and 25 µL of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide or MTT(Sigma,German) at concentration 5mg/ml were added to each well and after 4 hours optical densitys were readed using a microplate reader (Novin Gostar,Iran) at 490 nm and cell death percentage of each group was measured.

-The study of LDH leakage:

LDH activity was calculated by a non-radioactive assay using the LDH kit (Pars Azmun, Iran). The LDH test is based on the LDH release from cells with injured cellular membranes. Hence, in cell medium, the course of nanoparticles cytotoxicity can be quantified by calculating the activity of LDH in the supernatant. Briefly, after 24 incubation, reagent 1 and 2 that contain lactate and NAD were mixed and 10 microliter of cell supernatants were added to them. The mean optical densities at 340 nm were calculated in 5 minutes and LDH quantities were measured according to standard curve.

-The study of cell metabolic and cell ATP content:

After incubation and washing with HBSS , 100 µL of RPMI1649 media with 1% L-glutamine and 25 µL of the alamarBlue reagent (Invitrogen,UK) was added to each well and incubated 4 h. Cell metabolic was measured by reading optical density at excitation

544 nm and emission 590 nm using microplate reader(Novin gostar,Iran) and for ATP ,100 µL of ATP Determination reagent(Invitrogen,UK) was added and incubated 10 min until luminescence signals was stable then ATP was quantified by luminometer (Turner Biosystems, model 9100-102).

- Processing of Data and statistics:

All tests were done with 10 Balb/c mice and each were repeated two times and the results were showed as the mean plus or minus the standard deviation. The Levene test was carried out for determination of variances homogeneity and parametric tests (Student's t-test) were used to evaluate the results for significant differences. For data analyzing,SPSS software (v 16.0 for Windows; SPSS Inc.) was used and $P<0.05$ values were considered statistically significant.

Results

Structure, size and composition of nanoparticles:

Table 1 and 2 showed that lectin coated nanoparticle have larger size in DW and bigger size in PBS than naked (without lectin coating) nanoparticles. The SEM images confirmed this findings, too. FTIR spectrum analysis of all lectin coated nanoparticles revealed the presences of amide band I (1635 cm−1), amide band II (1535 cm−1) and amine group band (3300-3500 cm−1) that indicated lectin has been adsorbed by nanoparticles. Also, UV-VIS spectrum was showed decreasing of optical density of lectin solution at 280 nm that indicated protein adsorption, too.

Table 1. The size distribution of nanoparticles in DW

NPs	DLS results	Lectin coated NPs	DLS results
TiO2 NPs	10-25 nm	**Lectin coated TiO2 NPs**	15-30 nm
ZnO NPs	10-30nm	**Lectin coated ZnO NPs**	15-35 nm
MgO NPs	30-40nm	**Lectin coated MgO NPs**	35-40 nm
Ag NPs	20-30 nm	**Lectin coated Ag NPs**	25-35 nm
Au NPs	20-30 nm	**Lectin coated Au NPs**	25-35 nm

Table 2. The size distribution of nanoparticles in PBS

NPs	DLS results	Lectin coated NPs	DLS results
TiO2 NPs	100-500 nm	**Lectin coated TiO2 NPs**	10-100 nm

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ZnO NPs	100-500 nm	**Lectin coated ZnO NPs**	10-100 nm
MgO NPs	200-1000 nm	**Lectin coated MgO NPs**	20-100 nm
Ag NPs	100-1000 nm	**Lectin coated Ag NPs**	10-100 nm
Au NPs	100-1000 nm	**Lectin coated Au NPs**	10-100 nm

MTT and LDH results:

The MTT and LHD results are showed in Figure1 (a,b,c,d and e) and Figure2 (a,b,c,d and e) and these results can be extracted from these charts :

1. There was a direct relationship and significances differences between nanoparticle concentrations and related cell death percentage and LDH leakage (0.05>p). This was observed for both naked and lectin coated nanoparticles at dark and with UV/IR exposure. The most cell death and LDH leakage in all groups was as follows: 1000 mg/L > 100 mg/L > 10 mg/L > 1 mg/L ≈ 0.1 mg/L ≈ 0.01 mg/L for all groups. This means that there are no effect at concentration between 1 mg/L to 0.01 mg/L.

2. Among different groups that were studied , the most cell death and LDH leakage was as follows:

Nanoparticles+UV>Nanoparticles+IR>Nanoparticles>Lectin coated Nanoparticles+UV> Lectin coated Nanoparticles+IR> Lectin coated Nanoparticles. There was a significances differences between this groups(0.05>p).

This result was true for all nanoparticles (naked and lectin coated) used in this research. The results showed that the use of UV and IR exposure lead to increasing of nanoparticle cytotoxicy and lectin coating nanoparticles lead to decreasing of nanoparticle cytotoxicy.

3. both UV and IR irradiation can induce cytotoxic effect without nanoparticles but UV/IR have synergy with nanoparticles and lead to more toxicity effect. It has also been shown that UV irradiation has more effective than IR irradiation.

4. Among different forms that were studied, the most effective nanoparticle was as follows: AgNPs > AuNPs > TiO2NPs > ZnONPs >MgO NPs .This difference was seen in all groups(with lectin coated and with irradiation) with 0.05>p .

5. No significances difference in cell death and LDH leakage was observed in different mice cells (0.05<p).

-The study of cell metabolic function and cell ATP content:

Figure 3 and figure 4 show cell metabolic function and cell ATP content after 24 incubation with nanoparticles and UV/IR exposure. This tests confirmed the MTT and LDH results and indicated the use of UV and IR exposure with and without nanoparticles lead to decreasing of cell metabolic function and cell ATP content but and lectin coating nanoparticles lead to increasing of cell metabolic function and cell ATP level. Among different forms that were studied, the most effective nanoparticle that affect the quantity of cell metabolic function and cell ATP level, was as follows: AgNPs > AuNPs > TiO2NPs >

ZnONPs >MgO NPs. In all groups at concentration between 10 mg/L - 0.01 mg/L , there were no effect on the quantity of cell metabolic function and cell ATP level.

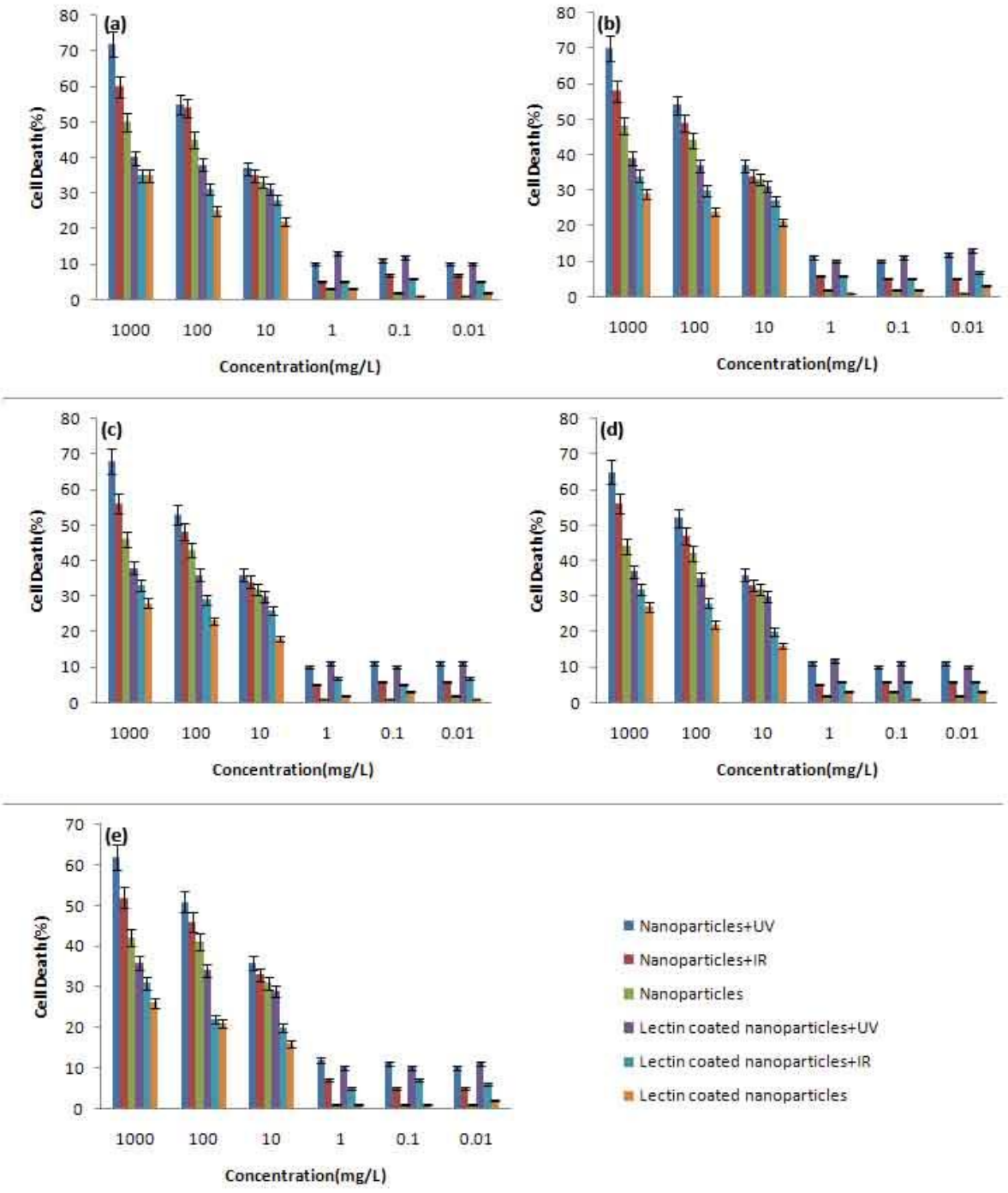

Figure 1. Cell death percentage induced by Ag Ag NPs(a) , Au NPs (b) , TiO2 NPs (c) , ZnO NPs (d) , MgO NPs (e) and lectin coated of these nanoparticles at dark condition and with UV and IR exposure

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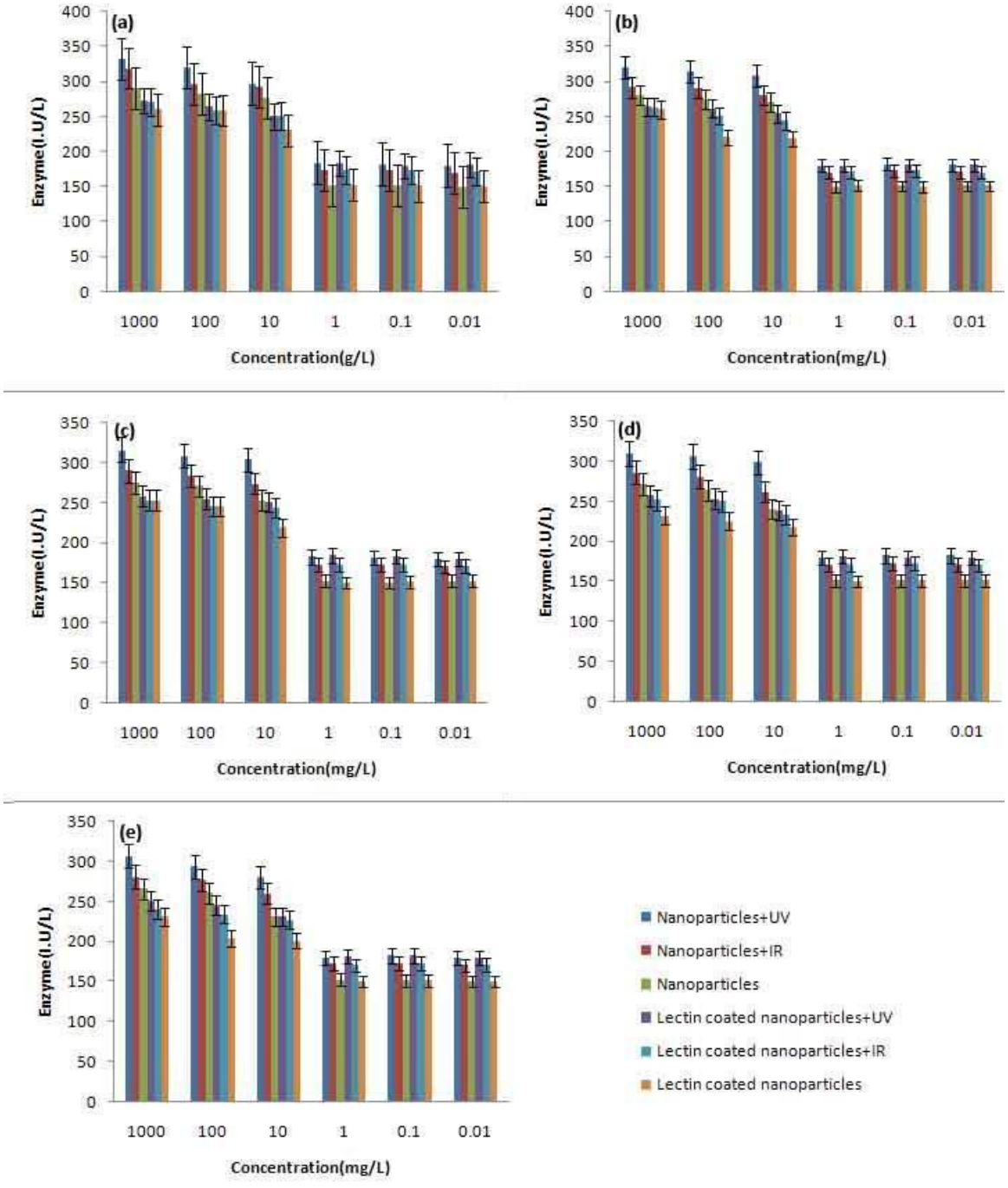

Figure 2.LDH leakage induced by Ag NPs(a) , Au NPs (b) , TiO2 NPs (c) , ZnO NPs (d) , MgO NPs (e) and lectin coated of these nanoparticles at dark condition and with UV and IR exposure

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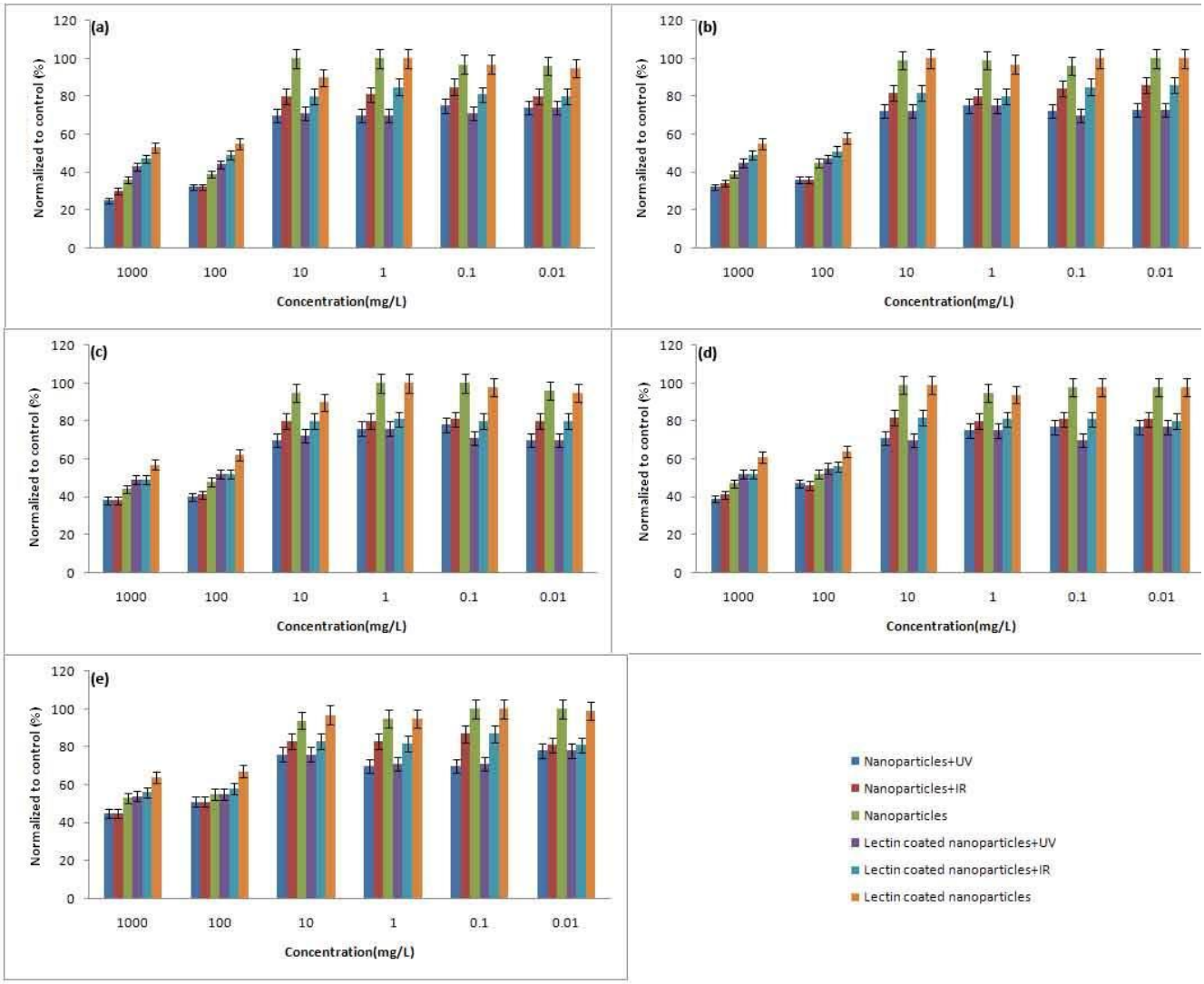

Figure 3. Cell metabolic affected by Ag NPs(a) , Au NPs (b) , TiO2 NPs (c) , ZnO NPs (d) , MgO NPs (e) and lectin coated of these nanoparticles at dark condition and with UV and IR exposure

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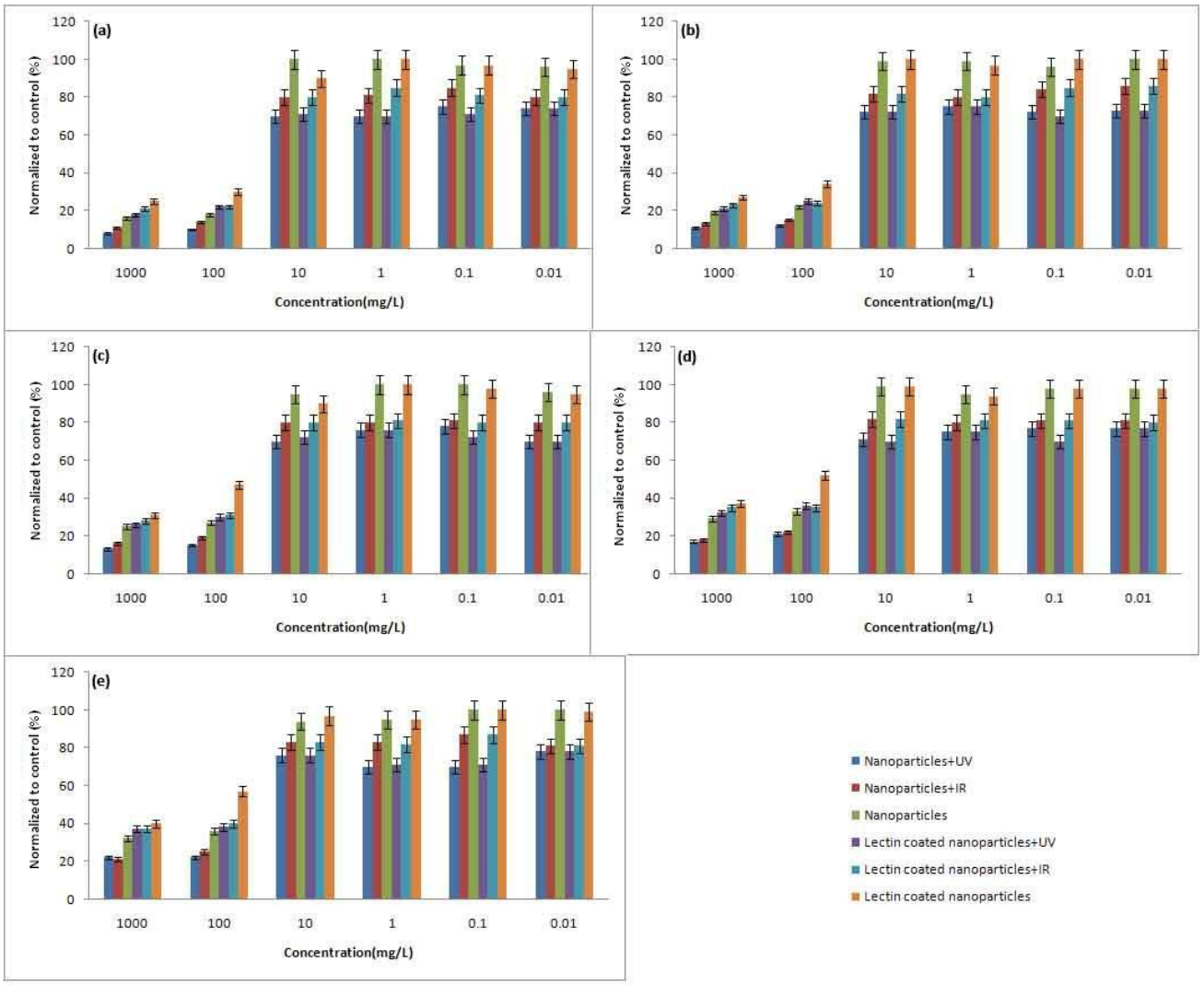

Figure 4. ATP level affected by Ag NPs(a) , Au NPs (b) , TiO2 NPs (c) , ZnO NPs (d) , MgO NPs (e) and lectin coated of these nanoparticles at dark condition and with UV and IR exposure

Discussion:

In this research, cytotoxicity of TiO2 NPs, ZnO NPs, MgO NPs, Ag NPs ,Au NPs and lectin coated of these nanoparticles with UV and IR irradiation on different cells(skin, macrophage, heart, liver and kidney) of Balb/c mice were measured by MTT assay , LDH

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enzyme release, quantity of cell metabolic function and cell ATP level. In this study, metal(Au and Ag) nanoparticles and metal oxide(TiO2 , ZnO and MgO) nanoparticles were used because of wide usage and application of these nanoparticles in medicine for treatment, detection and cell imaging(1-5). previous studies were showed that the effect of nanoparticle interactions are depend on their chemical composition, size , surface modification and the use of electrical, magnetical or electromagnetic waves together nanoparticle (20-24). For example, some nanoparticles are creating ROS that have the capability to devastate cells after UV light irradiation and generate hydroxyl radical and superoxide anion (20-22). On the other hand , some nanoparticles like AuNPs with NIR light can induce photothermal effect. Such nanoparticles absorb the energy and convert to heat which increases the temperature and take cells away by breaking the membrane (23-24).

Surface modification is another important parameter that affect nanoparticle cytotoxicity DNA and protein can bind covalently or non- covalently on nanoparticle surface (30) and thereby, generating a protein or DNA corona on nanoparticle surface through with a dynamic process. Protein adsorption to the nanoparticle surface can alter nanoparticles cytotoxicity and lead to uptaking of nanoparticles via receptor-mediated endocytosis (25-28). Stable peptide or protein conjugates of nanoparticle can be prepared by passive adsorption due to hydrophobic and electrostatic forces between nanoparticles surface and protein or peptide. This process is maximally done at a pH near the pI of the coated protein. The importance of this phenomenon is when different concentration of protein bound to the nanoparticles. In low protein adsorption the aggregation occurs, while added electrolytes to nanoparticle suspension (31-32). Uptak of nanoparticles, coated serum protein, via receptor-mediated endocytosis is a new research field in nanotoxicology (33-

34). This phenomen is not clear when there is no or low receptor on the surface of the target cells and do endocytosis occurs or not? and do affect nanoparticle Cytotoxicity? Lectins are carbohydrate -binding proteins that are highly specific for their sugar moieties. For example, PNA is one important lectin that extract from Arachis hypogaea and bind to Galβ1-3GalNAcα1-Ser/Thr (T-Antigen). The role of lectin is in biological recognition involving proteins and cells. For example, viruses use lectins to bind themselves to the host cells during infection. lectins may bind to a soluble suger or to a carbohydrate moiety that is a part of a protein or lipid(35). In this study the cytotoxicity of lectin coated nanoparticle was evaluated on different mouce cells like skin, macrophage, heart, liver and kidney.

This research showed that lectin coated nanoparticle have larger size than naked nanoparticles in DW because protein adsorption on the nanoparticle surface and forming a protein corona but lectin coated nanoparticle have smaller size than naked nanoparticles in PBS because of nanoparticle aggregation by electroestatic forces induced by PBS medium salt ions such as Na2H2PO4, Na2HPO4 ,NaCl and KCl (36).

FTIR spectrum of all lectin coated nanoparticles showed amide band I ,amide band II and amine band that indicated the presence of lectin layer and noted in other studies (30-32). Also, UV-VIS spectrum was confirmed the FTIR test and showed decreasing of optical density of lectin solution at 280 nm that indicated protein adsorption(25-28).

There are a direct relationship between nanoparticle concentrations and related cell death, LDH leakage, cell metabolic function and cell ATP level in all condition (dark, UV and IR exposure) and the most cell death and LDH leakage were as follows: 1000 mg/L > 100 mg/L > 10 mg/L > 1 mg/L ≈ 0.1 mg/L ≈ 0.01 mg/L but the most cell metabolic function and

cell ATP level were 1000 mg/L < 100 mg/L < 10 mg/L ≈ 1 mg/L ≈ 0.1 mg/L ≈ 0.01 mg/L. This phenomen is dose dependent that is common in toxicology studies and observed in other research (37).

Also, this study showed that the most cell death and LDH leakage for each nanoparticle were as follows: Nanoparticles+UV > Nanoparticles+IR > Nanoparticles>Lectin coated Nanoparticles+UV> Lectin coated Nanoparticles+IR> Lectin coated Nanoparticles but the most cell metabolic function and cell ATP level were Nanoparticles+UV <Nanoparticles+IR < Nanoparticles< Lectin coated Nanoparticles+UV< Lectin coated Nanoparticles+IR< Lectin coated Nanoparticles.

This results showed firstly, that the use of UV and IR exposure lead to increasing of coated and naked nanoparticle cytotoxicity This finding is hypothesized that UV irradiation generate free radicals and IR irradiation generate heat and increase medium temperature according to pervious studies(20-24). Both free radicals and heat lead to damage cells and increasing nanoparticle cytotoxicity. Another finding is that UV irradiation with nanoparticles is more effective and more synergistic than IR irradiation. Probably, because of free radical impact, generated by UV and lead to releasing of nanoparticle atoms and increasing cytotoxicity. Controversy, Lectin coated nanoparticles lead to decreasing of nanoparticle cytotoxicity. This means lectin coated nanoparticle can not distrust and damage cells as well as naked nanoparticles. The reason of greater effect of naked nanoparticles can be due to protective shielding and limitation of direct interaction between the nanoparticle and cell organelles, thus lead to low toxicity effect of nanoparticles.(38)

The most effective nanoparticle was as follows: AgNPs > AuNPs > TiO2NPs > ZnONPs >MgO NPs .This difference was seen in all groups (with lectin coated and/or irradiation) that this indicated Ag and Au nanoparticles are more cytotoxic than metal oxide nanoparticles(TiO2 ,ZnO and MgO). Interestingly, this nanoparticle with UV/IR irradiation or with lectin coating are more cytotoxic.

The authors hypothesized that different influence, structure and chemical composition of metal and metal oxide nanoparticles can be affected the high toxic effect of metal oxide nanoparticles. Since the experimental conditions like incubation time, cells , media and nanoparticle size are different between other researchs (6-8), therefore, comparison between these studies can be done hardly.

Another finding related to this research is that no cytotoxic difference was observed between different mice cells by coated or naked nanoparticles. It is means that naked nanoparticles and lectin coated nanoparticles have no certain target on cells and don't prefer specific cells. Other studies showed that nanoparticles can be binded to all compartment of cells such as membrane lipid/protein(39)

Conclusion:

The UV and IR exposure lead to increase of metal and metal oxide nanoparticle cytotoxicity and lectin coated nanoparticle lead to decreasing of nanoparticle cytotoxicity on Balb/c mice cell.

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