World Journal of Pharmaceutical ReseaRch

Volume 3, Issue 1, 765-784. Research Article ISSN 2277 – 7105

ANTI-CANCER EFFECT OF NANO PALLADIUM-LIPOIC ACID COMPLEX FORMULATION AGAINST LUNG CANCER CELL LINE

Kanchana M*, Savitha D[a] & Jeevitha D[a]

[a]*Associate Professor [a]Research Scholars, [a]Department of Biochemistry, Sathyabama Dental College and Hospitals, Sathyabama University, Rajiv Gandhi salai, Chennai-600119, Tamilnadu, India.

Article Received on
16 October2013
Revised on 19 November 2013,
Accepted on 12 December 2013

***Correspondence for Author:**
Dr. M.Kanchana
Associate Professor
Department of Biochemistry, Sathyabama Dental College and Hospitals, Sathyabama University, Rajiv Gandhi salai Chennai, India.
kanchibms@yahoo.co.in

ABSTRACT

The ever-increasing use of plant-derived phytochemicals and thiol rich molecules for the synthesis and stabilization of non-toxic metallic nanoparticles has gained increasing momentum for anti-cancer drug design and bioavailability. In this work, we synthesised a nanoformulation using *Solanum trilobatum* (SN) phytochemicals and dihydrolipoic acid (LA) for the production of palladium nanoparticles (PDNPs). The synthesized nanoparticles were analyzed by TEM. Further, this study evaluates the therapeutic efficacy of SNPDNPs and SNPD-LANPs in human lung (A549) cancer cells including cell viability comparison, cell morphology analysis and oxidative stress parameters. Accordingly, SNPD-LANPs exhibited significant cytotoxicity in A549 cells in a dose-dependent manner compared to SNPDNPs as determined by MTT and LDH analysis. SNPD-LANPs and SNPDNPs treatment also revealed significant ($P<0.05$) decrease in intracellular enzymatic and non enzymatic levels and increased lipid peroxidation status (TBARS) when compared to untreated A549 cells. Our result was also demonstrated a considerable alteration of apoptotic indices (nuclear morphology change stained with DAPI) as viewed through fluorescence microscope in SNPD-LANPs than SNPDNPs treated cancer cells. Taken together, the results of the present study which demonstrates that SNPD-LANPs might induce metabolic manipulation that significantly generates cell death via apoptosis and necrosis without affecting the normal cells.

Keywords: Antioxidant enzymes, lipoic acid, oxidative stress, palladium nanoparticles, *solanum trilobatum*.

1. INTRODUCTION

Lung cancer is still a commonly diagnosed cancer as well as the leading cause of cancer death in males globally. Among females, it was the fourth most commonly diagnosed cancer and the second leading cause of cancer death [1]. Present therapy such as surgery, radiotherapy, and chemotherapy are mostly unsuccessful against advanced stages of cancer, and are also often associated with severe side effects [2]. To overcome these side effects in cancer therapy, efforts have been directed to search for methods to develop therapeutic modalities with no or minimal side effects to normal organs.

Progress in therapy of cancer has also received gifts from the development of nanotechnology and metallic nanoparticles uses increased for their potential efficacy [3]. Metallic nanoparticles (like silver, platinum, gold and palladium) [4, 5] are the emerging nanoproducts that have gained interest in the field of nanomedicine due to their unique properties and obvious therapeutic potential in treating a variety of diseases. Nanoparticles improve the solubility of poorly water-soluble drugs, modify pharmacokinetics, increase drug half-life by reducing immunogenicity, increase particularity towards the target cell or tissue (therefore reducing side effects), improve bioavailability, diminish drug metabolism and enable a more controllable release of therapeutic compounds and the delivery of two or more drugs simultaneously for combination therapy [6-8]. Among the nanoparticles, palladium nanoparticles are having an extensive application in heterogeneous and homogeneous catalysis due to their high surface to volume ratio [9-11]. Synthesis of PDNPs using chemical reduction technologies are well explored but, as it involves extreme operational conditions like high temperature, pressure and numerous toxic chemicals this has become a non eco-friendly and high cost technique [12].

Of late, extensive research effort has been made in utilizing various biological systems and plants that was found to be simple and versatile process for the synthesis of different types of metal nanoparticles [13-15]. Among them plant extract mediated biological process was found to be simple and versatile process for the synthesis of different types of metal nanoparticles such as silver, gold and palladium, which has emerged as an alternate to conventional physical and chemical methods [16-22]. Reports have shown that the leaf

extracts of Diopyros kaki [22], *C. Camphora* [23] , extracts from the bark of *Cinnamon zeylanicum* [24] , extracts from the tuber of *Curcuma longa* [12] and extracts from the peel of banana [25] played a role as reducing and stabilizing agents for the synthesis of palladium nanoparticles.

In addition, the presence of the free radical scavenger, lipoic acid [26] and the addition of an alternative energy source palladium [27], led to consider that palladium-lipoic acid complex (LAPd) as a non-toxic chemotherapeutic agent for the treatment of various cancers [27,28]. Ramachandran and his co-worker in their study, they have complexed silver nanoparticles with lipoic acid by means of a surface stabilizing agent, Pluronic F 127 and confirmed the feasibility in using the same as a therapeutic adjuvant during cancer radiotherapy [29]. In continuation with our previous investigation [30], an attempt was made to synthesize palladium nanoparticles using the polyphenols, present in *Solanum trilobatum*, an anti-cancer herb (*solanum*-nanopalladium-SNPD) and α-lipoic acid (*solanum*-nanopalladium lipoic acid-SNPD-LA) as stabilizing agents. However, the primary toxicity and distribution of SNPDNPs and SNPD-LA nanoparticles remains unexplored. Here, an effort has been put forth to understand various steps in palladium nanoparticles toxicity by studying the effect of polyphenols of *Solanum trilobatum* and lipoic acid coated palladium nanoparticles on cell viability, biomarkers for oxidative stress such as glutathione (GSH), malondialdehyde (MDA) and lactate dehydrogenase (LDH) and chromosomal aberrations in human lung cancer cell line (A549).

2. EXPERIMENTAL METHODS

2.1.Synthesis of palladium nanoparticles from *Solanum trilobatum* leaf extracts (SNPDNPs)

The broth used for reduction of $PdCl_2$ ions to palladium was prepared by taking 20g of thoroughly washed and finely cut *Solanum trilobatum* leaves (from local market, Chennai) in a 500 ml Erlenmeyer flask with 100 ml sterile distilled water and then boiling the mixture for 1 min. The process of boiling the leaves leads to rupture of the walls of leaf cells and thus, release of intra-cellular material into solution. After boiling, the solution was decanted, filtered and 5ml of this broth was added to 100 ml of 0.226 M $PdCl_2$ aqueous solution.

2.2. Palladium nanoparticles from *Solanum trilobatum* leaf extract conjugated with Lipoic acid (SNPD-LANPs)

15 ml of the leaf extract was taken and added 0.226M $PdCl_2$ aqueous solution with 1 ml of lipoic acid (Sigma corporation) (15mg lipoic acid was dissolved in 0.5ml of ethanol and 0.5ml of water) and kept for incubation at room temperature for 24 h. The mixture was centrifuged at 4,500 rpm to separate the capped PDNPs. The resulting palladium nanoparticles were purified by repeated centrifugation at 15,000 rpm for 20 min, with the pellet produced by this process redispersed in deionised water. The obtained nanoparticles were subjected to characterization for their stability (UV-spectroscopy), functional group analysis for confirming their involvement in forming a nanocomplex (FTIR) and SEM for characterizing the morphology and size in our previous study [30]. Additionally, in this study, we have performed TEM to confirm the morphology of the obtained nanocomplexes.

2.3. Cell culture

The human broncho alveolar carcinoma-derived cell line (A549) was purchased from Pune, NCCS. Cells were maintained in Hams F-12 medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100μg/ml streptomycin, and grown at 37°C in a 5% CO_2 humidified environment. The test solution of SNPDNPs and SNPD-LANPs was prepared in the culture media and dispersed for 20 min by using a sonicator to prevent aggregation. The cells were treated with various concentrations of particles, and a time schedule is designated in the following section of each toxicological study.

2.4. Cell viability test

Cell viability was measured by the MTT (3-(4-5-dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide (Sigma) assay. Cells were seeded on 96-well tissue culture plates with 5×10^3–2×10^4 cells in 100 µl media per well. Cells were treated with 10, 40, 80, 120 & 160µg/ml concentrations of SNPDNPs & SNPD-LANPs complexes for 24 and 48 h respectively. At the end of exposure, 40µl of MTT solution (2 mg/ml) was added and the cells were incubated for 4h at 37 °C. Cells were treated with 150µl of DMSO and absorbance was quantified in 540 nm using the micro plate spectrophotometer system (VersaMax, Molecular Devices, and Sunnyvale, CA, USA). The viability of the treated group was expressed as the percentage of control group that was assumed to be 100%. The conversion of lactate to pyruvate was detected using the Cytotoxicity Detection Lactate Dehydrogenase kit (Roche Applied Science, IN, USA) following the manufacturer's instructions. The cells

treated with SNPDNPs & SNPD-LANPs complexes were washed twice with ice-cold PBS, harvested by centrifugation at 250 g for 10 min at 25°C. The supernatant obtained was used for determining the LDH activity. Optical densities resulting from LDH activity were measured in a micro plate reader at 490 nm. Results were given as mean ± standard deviation of three independent experiments.

2.5. Estimation of oxidative stress parameters

The human lung cancer cells (A549) were cultured and exposed to SNPDNPs and SNPD-LANPs at the concentration of 60µg/ml for 24 hours. These cells were washed and harvested in ice cold PBS. The harvested cells were then lysed in cell lysis buffer (20mm Tris-HCl [pH 7.5], 150mm NaCl, 1mm Na_2EDTA, 1% Triton and 2.5mm sodium pyrophosphate). The lysed cells were then centrifuged and the supernatant was used for the further assays of biomarkers of oxidative stress. The extent of Lipid Peroxidation (LPO) was determined by the formation of pink chromogen when lipid peroxides reacted with thiobarbituric acid (Thiobarbituric acid-2-malondi- aldehyde adduct), and was measured at 532 nm against blank using spectrophotometer. The Thiobarbituric Acid Reactive Substance (TBARS) measured was expressed as n moles of MDA formed/min/mg protein in samples [31]. The activity of SOD was expressed as Units/mg of tissue protein and was assayed by monitoring the inhibition of the reduction of nitro blue tetrazolium by the sample at 560nm [32]. The activity of CAT was examined as the rate of decrease in the absorbance of H_2O_2 at 240nm/min/mg protein [33]. The activity of GPx in cell extracts was expressed as nmoles of CDNB conjugated/min/mg protein. One unit of enzyme activity is the amount of the enzyme that converts 1 µmole of GSH to GSSG and recycling of GSSG by GR in excess in the presence of H_2O_2/min [34]. The amount of Protein in the samples was estimated by the method of Lowry *et al*., 1951 [35]. The GSH content was determined according to the method of Akkemik, *et al*., 2011, based on the reaction with 5,5'-dithio-bis (2 nitro benzoic acid) (DTNB or Ellman's reagent) which gave a yellow colour that was measured at 412 nm [36].

2.6. Analysis of nuclear morphology change

The cells were treated with 60µg/ml of SNPDNPs & 60µg/ml of SNPD-LANPs, and then washed once in phosphate buffer saline (PBS) followed by fixation in cold methonal: acetone (1:1) for 5 min. After washing thrice in PBS for 5 min, these cells were treated with 4µg/ml 4′, 6-diamidine-2′-phenylindole dihydrochloride (DAPI) (Sigma) for 10 min at room temperature. Nuclei of the treated and control cell lines were stained with DAPI and

visualized using an Olympus fluorescence microscope equipped with an Olympus Cool Snap Camera (Tokyo, Japan).Cells were randomly selected for examination at a high magnification (×40) and photographed.

2.7. Statistical analysis

Data represent the mean + SD of triplicates from three independent experiments. Statistical differences were obtained using the analysis of variance, and the Dunnett's and Turkey's tests (SPSS v. 12 programs).

3. RESULTS AND DISCUSSION

The synthesis of nanoparticles of specific composition and size is a promising area in materials science research. New routes to the manufacture of these materials extend the choice of properties that can be obtained from nanoparticles. Though, the palladium lipoic acid complex is one of the best investigated drugs for their toxicity, there is a paucity of data on nanopalladium-lipoic acid complex. The phyto-synthesis of palladium nanoparticles offers a new means to develop environmentally benign nanoparticles [37] and also has been briefly discussed in our preliminary study [30]. The water-soluble fractions comprised of complex polyols in the biomass were believed to have played a major role in the bioreduction of palladium ions [38]. Furthermore, the stability of palladium nanoparticles can be attributed to the formation of stable bonding between metallic palladium and reducing sugars, the phytochemicals such as saponins, tannins, terepenoids, flavonoids, anthocyanins, betacyanins, proteins and steroids and phenolic acids present in the leaf broth of *Solanum trilobatum* [39].

The modification of the surface of the metallic nanoparticles with some functional groups, such as cyano, thiol [40], glutathione [41], and amino groups [42,43] are known to be very stable, biodegradable, biocompatible and with high specificity. In the present study, the disulfides in lipoic acid are reduced by the palladium chloride ions to two thiol groups, which may be involved in the binding of lipoic acid to SNPDNPs [44]. This enabled the weaker PD-SN bonding interactions, which can easily break from the surface of the Pd nanoparticles during a longer reaction time [45]. Studies have evidently illustrated that glutathiones (a thiol rich molecule) used for capping gold quantum clusters (AU-n-SG-m) (-SG, glutathione thiolate) has been well known for the stability of the AUNPs synthesized chemically [44]. In addition, the binding of such thiol-rich molecules is that they home in to the tumour vasculature, which is less dependent on the variability of receptors expressed directly on the

tumour cell surface [46] thereby developing a rapid release without appreciably contributing to drug loss during circulation in the central blood compartment. One of our previous studies have shown the cytotoxicity and oxidative stress caused by lipoic acid and grape phytochemicals stabilized gold nanoparticles in human breast cancer cells [47]. This prompted us to investigate the toxicity of solanum palladium nanoparticle (SNPDNPs & SNPD-LANPs), which constitutes palladium, lipoic acid and phytochemicals of *Solanum trilobatum*.

We explored the toxic responses of nanopalladium complexes to A549 cells to validate the commercially available palladium lipoic acid complex. The effects of nanoparticles on cellular processes mainly depend on their size, chemical composition, crystalline, and aggregation property. Consequently, we performed TEM (Fig. 1a and 1b) analyses to show the size and morphology of the obtained nanopalladium complexes. The TEM image represented the polydispersity of the formed palladium nanoparticles that are more or less spherical in shape with a size ranging between 40 to 70 nm. Therefore, for a nanoparticle to find their application, their shape plays a significant task. Our findings confirm with the study of Yong and his co-workers [48] who have reported that the PDNPs synthesized from *Desulfovibrio desulfuricans* had an average size of 50 nm. Also the work by Mallikarjuna *et al*., 2008 [49] who generated a green approach for silver (Ag) and palladium (Pd) nanoparticles using coffee and tea extract resulted with sizes ranging from 20-60 nm.

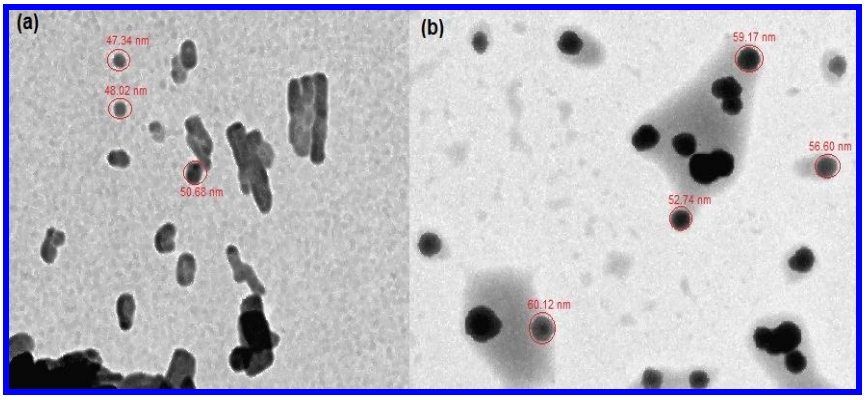

Fig. 1. TEM microscopic images of (1a) SNPDNPs & (1b) SNPD-LANPs

3.1. Effect of SNPDNPs & SNPD-LANPs complexes on Cell Viability and Cytotoxicity of A549 cells

In our present investigation, the foremost challenge in this study is to demonstrate the cytotoxicity of solanum nano-palladium lipoic acid complexes under *in vitro* conditions in

A549 cells using two cytotoxicity markers, including mitochondrial function (MTT reduction), and membrane integrity (LDH leakage). These are the basic markers for the estimation of toxicity for a wide range of nanoparticles in different kinds of cell lines, at which 50% of the cells die in a given time frame (LC50) [50]. To examine the toxic effects of SNPDNPs & SNPD-LANPs particles, A549 cells were incubated with different concentrations (10-160µg/ml) of nanoparticles for 24 and 48h and the cell viability was determined. After 48h, cell viability decreased as a function of nanoparticle concentration to 30% and 24.7%, upon exposure to 160 µg/ml of SNPDNPs & SNPD-LANPs complex respectively and cytotoxicity was not intensified by the increase in concentration up to160µg/ml (Fig. 2a). Results of MTT assays clearly revealed that SNPD-LANPs exerted slightly better cytotoxic effect than SNPDNPs in a dose-dependent manner towards A549 cells.

It is apparent that, the rate of glycolysis increases by cancer cells wherein there is conversion of pyruvate into lactate which catalyses by LDH, which consumes NADH and regenerates NAD. This in turn makes the cell more vulnerable to programmed cell death (apoptosis) and decreasing the cell viability indicating that cell death is the primary cause for the cell number reduction that is evident by the significant increase in the LDH activity and promoting cell membrane damage. The extent of A549 cell membrane rupture was revealed by LDH levels in cell medium. The LDH levels in the cell culture were increased in all treatment groups after exposure to SNPDNPs & SNPD-LANPs nanoparticles for a period of 48 h by 73.5% and 58.1%, respectively (Fig. 2b). The release of LDH in our study can be attributed to the excellent viability of the cells treated with SNPDNPs and SNPD-LANPs as proven by LDH assay. Nanopalladium complexes pre-treatment at a concentration of 160µg/ml reduced the LDH leakage to a minimum, and this concentration is used in subsequent studies. Both these assays demonstrated that the solanum nano-palladium lipoic acid complex exert significant cytotoxicity to A549 cells in dose-dependent manner in the concentration range of 10-160µg/ml. The noticeable cytotoxicity is due to the smallest size that can interact readily with biomolecules either on the surface of or within cells. The cytotoxicity results of the present study can be attributed to the cytotoxicity induced by chemically synthesized palladium lipoic acid in cancer cell lines like colon carcinoma (SW 620 and HCT 116), lung carcinoma (H 460), and breast carcinoma (MCF-7) [51]. In addition, Lin *et al*., 2006 established in his study that upon treatment with 15nm and 46nm of amorphous silica nanoparticles at 50µg/ml for 48 h, to A549, the cell viability decreased significantly but was same for both sizes and

found to be 76.0% of control [42, 52]. Thus, with nanoparticles toxicity, it is often expected that the smaller the size, the stronger the exerted cytotoxicity [53]. Therefore the sizes of our nanoparticles (40-70 nm) as evidenced by TEM hold promise in inducing cytotoxicity to lung cancer cells.

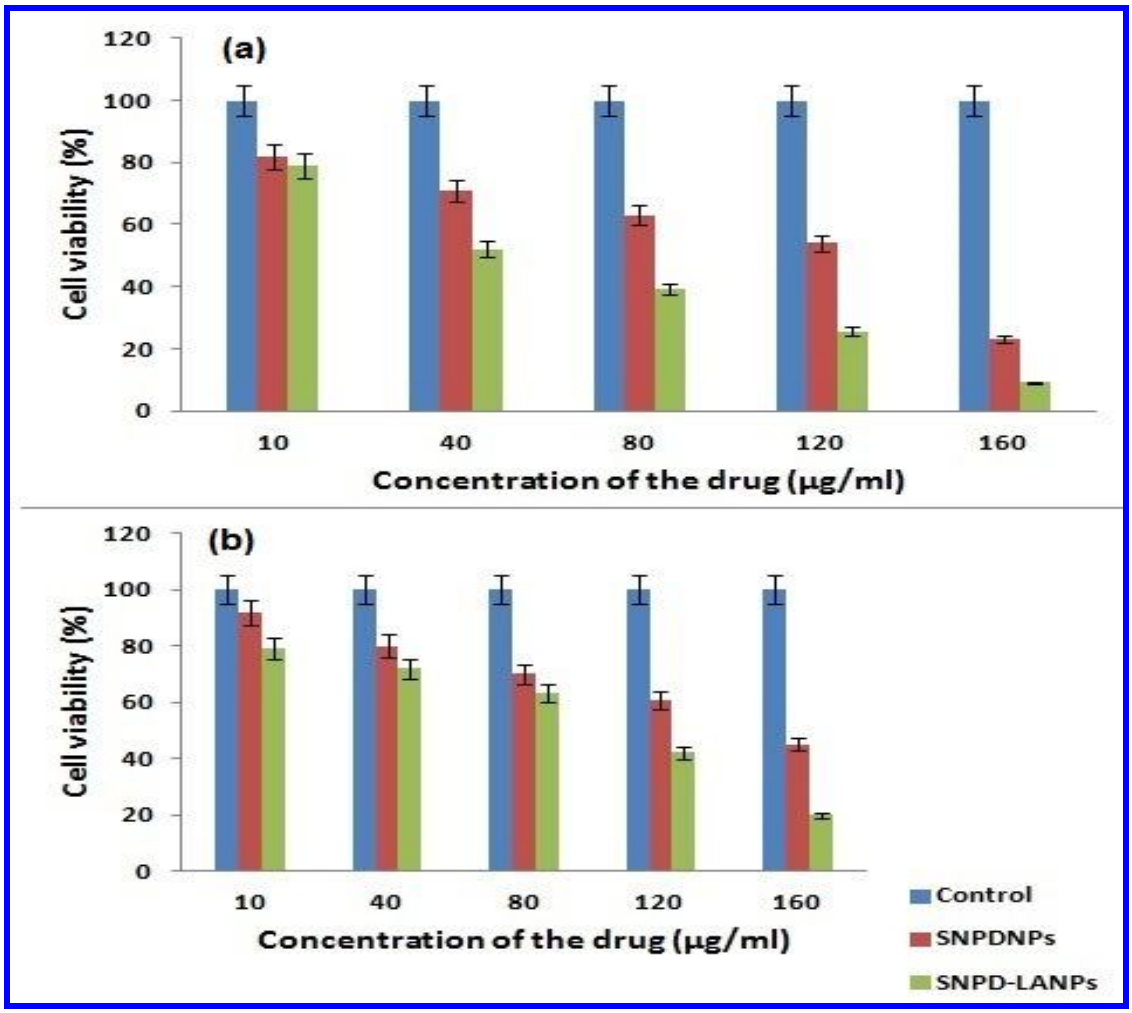

Fig. 2. (a) Cell viability of different concentrations of SNPD & SNPD-LANPs on A549 cells after 48h of treatment, as calculated from the MTT assay. (b) LDH release at different concentrations of SNPD & SNPD-LANPs on A549 cells after 48h of treatment. The values are represents the mean of three repeat for each treatment.

3.2. Effect of SNPDNPs & SNPD-LANPs complexes on antioxidant enzymes in A549 cells

PDLA complex is unique in electronic and redox properties that appear to be the key to their physiological effectiveness [27,54]. As previously mentioned, LAPD is not a single molecule but a complex and their liquid crystal structure thus provides a continuous stream of redox, which is novel to free radical biology. Thus, lipoic acid complexed with nanopalladium and

the phytochemicals of *Solanum trilobatum* have the ability to be a highly effective free radical scavenger and their ability to donate electrons to the mitochondria of the cell is critical in explaining their dramatic benefits. It was well documented that Oxygen Radical Absorbance Capacity (ORAC) analysis of palladium α-lipoic acid formulation demonstrates that it is approximately five times more potent antioxidant than α -lipoic acid and acts as both an exceedingly active free radical scavenger and alternative energy source to the vulnerable hippocampus of the brain [55]. Thus, the foremost biochemical changes in cancer cells after treatments with anti-cancer agents is the increase in ROS generation, which is frequently considered as a cancer-promoting factor [56].

One of the consequences of elevated oxidative stress is the production of malondialdehyde, an indicator of lipid peroxidation. Many studies have examined the possibility of an association between lipid peroxidation and cancer. It increases the peroxidation of PUFA in mitochondrial membrane and this effect along with cross-linking of proteins with amino groups of phospholipids and nucleic acids induced by MDA causes changes in membrane fluidity. Based on the results of cytotoxicity [57] and potential redox property of SNPDNPs & SNPD-LA nanoparticles we propose that the cells are adorned with oxidative stress. Since, LPO is a sensitive parameter for toxic effects of various environmental pollutants with oxidative properties [53]; the authors suspected that solanum palladium lipoic acid nanoparticles induced ROS might play a key role in LPO. Being exposed to SNPDNPs & SNPD-LANPs composites for 72 h, cellular MDA levels showed a significant ($p<0.05$) increase with the highest (60µg/ml) nanoparticle dosage level when compared to the control (Fig. 3a). This increased level was found to be highly significant in the cells treated with SNPD-LANPs when compared to the cells treated with SNPDNPs. MDA levels increased by 39.2%, and 49.1% after 72 h exposure to SNPDNPs & SNPD-LANPs composites respectively when compared to the control the groups. Further, LPO caused by exposure to nanosized particles has also been observed in other studies of nanoparticle toxicity [58]. Since the palladium-lipoic acid complex serves as a potent redox molecule, it may facilitate a chain breaking antioxidant effect on the lipid peroxidation process that may appear to be the key to their physiological efficiency [27, 28].

Cellular integrity is affected by oxidative stress when the production of ROS overwhelms antioxidant defence mechanism [59]. Toxic substances either suppress the activity of antioxidant enzymes or lead to an increased production of ROS, resulting in higher ROS

levels in the cell [60]. Cellular defence mechanism against superoxide includes a series of linked enzyme reaction to remove superoxide and repair radical induced damage. Catalase and glutathione peroxidase play an important role in the cellular protection from oxidative stress-induced cell damage. They catalyze the transformation of H_2O_2 within the cell to harmless by-products, thereby curtailing the quantity of cellular destruction inflicted by LPO products. Thus, CAT is a hemoprotein that requires NADPH for their regeneration to its active form and GPx is required to repair LPO initiated by superoxide in the phospholipid bilayer for maintenance of membrane integrity. Our results showing lower activities of SOD, CAT and GPx in cancerous lung cells are consistent with the general tendency of accumulation of H_2O_2, and O_2 in tumour cells [61]. In fact, a lower capacity to destroy H_2O_2 e.g., by catalase, peroxiredoxins, and GSH peroxidases may cause tumour cells to grow and proliferate more rapidly than normal cells in response to low concentrations of H_2O_2. It is well known that H_2O_2 exerts dose-dependent effects on cell function, from growth stimulation at very low concentrations to growth arrest, apoptosis, and eventually necrosis as H_2O_2 concentrations increase. Therefore in the present study, activity of SOD (30% and 62%), CAT (15% and 30%) and GPx, (39% and 56%) decreased upon exposure to SNPDNPs & SNPD-LANPs to A549 cells at LD_{50} concentrations, compared with untreated cells **(**Fig. 3b, 3c, 3d**).**

The changes in the activity of antioxidant enzymes on treatment of SNPDNPs & SNPD-LANPs may be attributed to the antioxidant effect and the chemoprotective role [62, 63] of the solanum phytochemicals such as isoflavonoids, phenols, phenolic acids, xanthones enriched in *Solanum trilobatum*. They reduce the free radical formation by decreasing the concentration of cytosolic iron, which plays an important role in oxygen radical formation. Besides, lipoate also has an effective role as a scavenger of hydroxyl, singlet, peroxide, and superoxide radicals [64] and sufficient evidence indicating the usefulness of the lipoic acid/dihydrolipoic acid redox couple as a therapeutic agent [64, 65]. Studies have also demonstrated that LA can regenerate or recycle the antioxidants CoQ (ubiquinol), vitamins C and E (via glutathione), and glutathione without itself becoming one in the process [66]. In addition, the capping agent, lipoic acid has the potential to increase glucose uptake in both *in vitro* and *in vivo* condition [67]. Enhanced glucose uptake by cells serves as a fuel for both pentose phosphate pathway and oxidative phosphorylation thereby bringing up the cellular levels of $NADPH/NADP^+$ which in turn alters the activity of CAT.

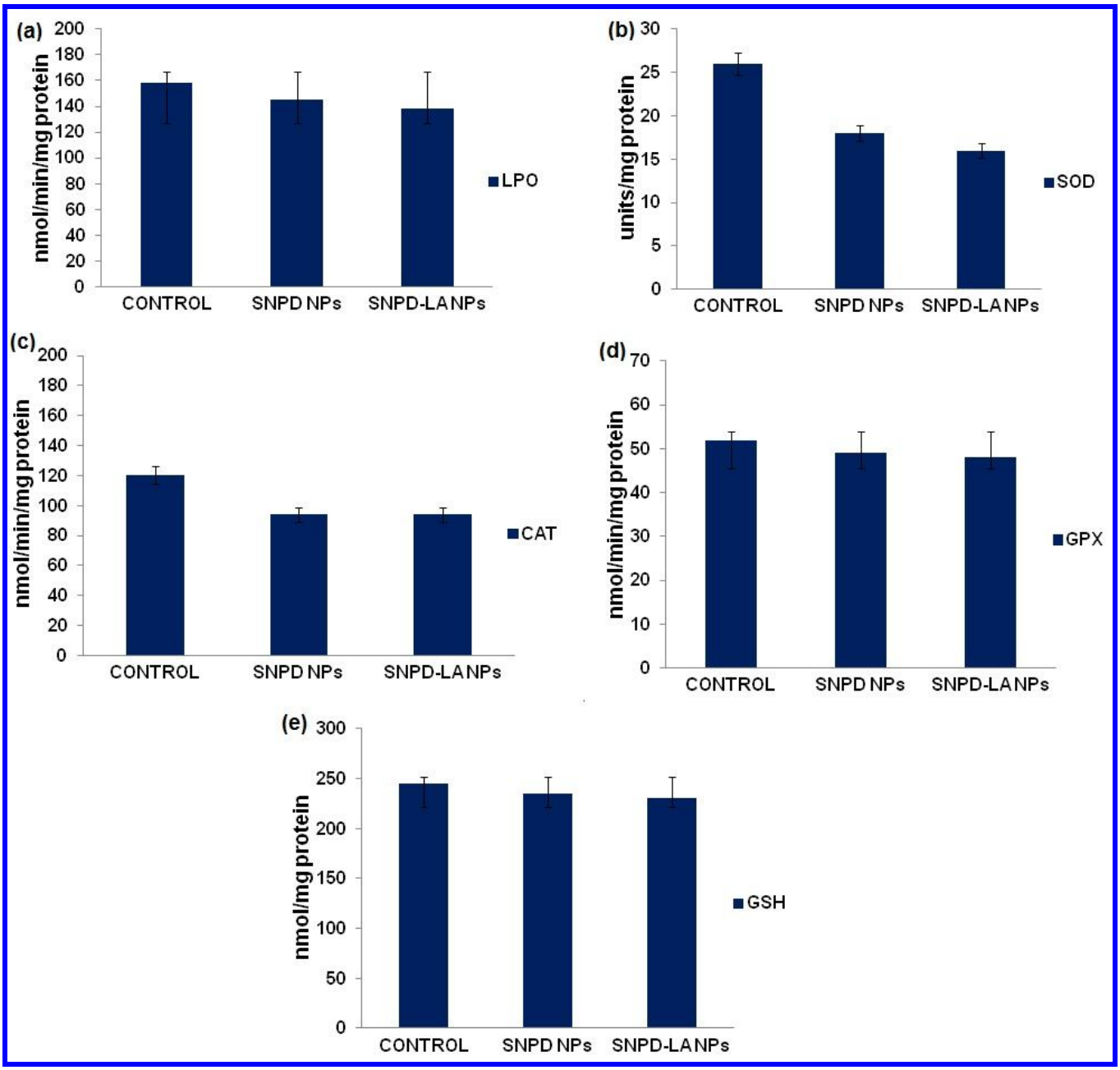

Fig 3. Effect of 60 µg/ml of SNPDNPs & SNPD-LA NPs in A549 cells on the levels of a) lipid peroxide (LPO), b) superoxide dismutase (SOD), c) catalase (CAT), d) glutathione peroxidase (GPx) and e) reduced glutathione (GSH) . The values are represents the mean of three repeat for each treatment.

With increased levels of NADPH, changes in the levels of GSH, a substrate for GPx may occur. GSH is vital to antioxidant defences, because besides serving as a substrate in the GPx reaction, it also acts as a free radical scavenger and helps regulate the thiol disulfide concentration of a number of glycolytic enzymes and $Ca2^{+}$-ATPases, thus indirectly

maintaining intracellular $Ca2^{+}$ homeostasis [68]. The patterns of the cellular GSH levels agree with the dose-dependent response observed in the cell viability study and a maximum decline ($P < 0.05$) of GSH level of exposure to 60µg/ml of SNPDNPs & SNPD-LANPs composites that ranged between 47.8% and 32.0% respectively is highlighted in this study (Fig. 3e). The observed decrease in GSH on treatment could be ascribed to the ability of lipoic acid to modulate cysteine availability, which is considered as the rate-limiting factor in their biosynthesis [65]. Sudheesh *et al*., 2010, in their study highligted the higher levels of antioxidant status (MnSOD, CAT, and GSHPx) with the PDLA treated groups of aged male rats group [69]. He also found a similar increase of the antioxidant enzymes levels, GSH and LPO levels alloxan induced diabetic group with the PDLA [70]. Although polyphenols of *Solanum trilobatum* [39] and LA display pro-oxidant properties under specific conditions, both compounds additionally act as strong antioxidants [71]. They function in various cell types to block or prevent oxidative stress-induced apoptosis but promote apoptosis in several cancer cell lines [72-77]. Thus it is evident that lipoic acid that acts as a capping agent in SNPDNPs could mitigate GSH consumption either by acting as an alternate scavenger of ROS or by increasing the levels of GSH by stimulating their biosynthesis.

3.3. Effect of SNPDNPs & SNPD-LANPs on Apoptosis in A549 cells

Induction of apoptosis in cancer cells is an important focus in the discovery of anticancer drugs. Recent research in cancer, propose that ROS mediated apoptosis is characterized by a series of morphological changes such as chromatin condensation, cell shrinkage, membrane blebbing, packing of organelles, formation of apoptotic bodies and inter-nucleosomal DNA fragmentation. Thus in our present study nuclear condensation events due to stress was found to be one of the signatures of cytotoxicity. Analysis of nuclear morphology change in the treated and untreated A549 cells was visualized at a magnification range of 40X using an Olympus fluorescence microscope. A minimum of 200 cells were counted and classified as follows: Both normal cells (Fig. 4**)** (with big nuclear, dispersion and homogeneous fluorescence) and the apoptotic cells (with nuclear shrinkage and hyper chromatic nuclei) were counted under each field. Kanchana et al., 2011 observed a similar pattern in breast cancer cells after treatment with phytochemicals and lipoic acid coated gold nanoparticles [47].

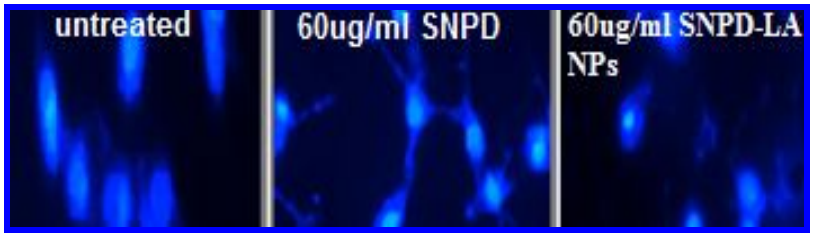

Fig. 4. Fluorescence micrographs of A549 cells stained with DAPI to visualize nuclear morphology. The untreated A549 cells contained round nuclei with homogeneous chromatin. The cells treated with SNPDNPs & SNPD-LANPs showed chromatin condensation, reduction of nuclear size, and nuclear fragmentation.

The cytotoxic effects of palladium are the result of active physicochemical interaction of palladium atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA**.** Interestingly, the polyphenolic antioxidants are scavengers of free radicals and modifiers of various enzymatic functions. Thus, phytochemicals of *Solanum trilobatum* coated on both the nanocomposites are closely associated with antioxidant properties that may be involved in the induction of A549 cell apoptosis. Moreover, among these molecules, alpha-lipoic acid has gained considerable attention since it was able to induce cell cycle arrest and apoptosis in different cancer cell lines [78], while it exerted protective effects in normal cells [72, 79]. Furthermore, as Lipoic acid, a vital ingredient in the SNPD-LANPs complex and an essential cofactor in the conversion of pyruvate to acetyl-CoA, helps the PD-LA complex to directly target the mitochondria where acetyl CoA is then channelled into the citric acid cycle to create NADH and $FADH_2$ to generate ATP, the cell's primary energy source.

In conclusion, the lipoic acid coated SNPDNPs might provoke metabolic manipulation that drastically generates cell death via apoptosis and necrosis without disturbing the normal cells making SNPD-LANPs a valuable entrant lead compound to contradict growing drug resistance in lung cancer.

CONFLICT OF INTEREST

The authors confirm that this article has no conflicts of interest.

ACKNOWLEDGEMENT

We are grateful to Sathyabama University for rendering us the good environment and facilities to complete this research.

REFERENCES

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E *et al.* Global Cancer Statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Feng SS, Chien S. Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chem Eng Sci 2003;58: 4087–114.

[3] Irena K. Platinum Complexes as Anticancer Agents. Recent Patents on Anti-Cancer Drug Discovery 2006; 1: 1-22.

[4] Watanabe K, Menzel D, Nilius N, Freund HJ. Photochemistry on Metal Nanoparticles. Chem Rev 2006; 106: 4301-20.

[5] Cheong S, Watt JD, Tilley RD. Shape Control of Platinum and Palladium Nanoparticles for Catalysis. Nanoscale 2010; 2: 2045-53.

[6] Nuria S, Pilar MM. Multifunctional nanoparticles properties and prospects for their use in human medicine. Trends in Biotechnol 2008; 2: 425-33.

[7] Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science 2004; 303: 1818-22.

[8] Emerich DF, Thanos CG. The pinpoint promise of nanoparticle-based drug delivery and molecular diagnosis. Biomol Eng 2006; 23: 171-84.

[9] Li Z, Wang X, Wen G, Shuang S, Dong C *et al.* Application of Hydrophobic Palladium Nanoparticles for the Development of Electro-chemical Glucose Biosensor. Biosen Bioelectron 2011; 26: 4619-23.

[10] Chen H, Wei G, Ispas A, Hickey SG, Eychmullar A. Synthesis of Palladium Nanoparticles and Their Applications for Surface-Enhanced Raman Scattering and Electrocatalysis. J Phy Chem C 2010; 114: 21976-81.

[11] Gopidas KR, Whitesell JK, Fox MA. Synthesis, Characterization, and Catalytic Applications of a Palladium-Nanoparticle Cored Dendrimer. Nano Lett 2003; 3: 1757-60.

[12] Sathishkumar M, Sneha K, Yun YS. Palladium Nanocrystal Synthesis Using Curcuma longa Tuber Extract. Int J Mat Sci 2009; 4: 11-17.

[13] Konishi Y, Ohno K, Saitoh N, Nomura T, Nagamine S, *et al.* Bioreductive Deposition of Platinum Nanoparticles on the Bacterium Shewanella Algae. J Biotechnol 2007; 128: 648-53.

[14] Pugazhenthiran N, Anandan S, Kathiravan G, Udaya Prakash NK, Crawford S, *et al*. Microbial Synthesis of Silver Nanoparticles by Bacillus sp. J Nanoparticle Res 2009; 11: 1811-15.

[15] Mohammed AF, Balaji K, Kalaichelvan PT, Venkatesan R . Fungal Based Synthesis of Silver Nanoparticles-An Effect of Temperature on the Size of Particles. Coll Surf B Biointerf 2009; 74: 123-26.

[16] Kumar V, Yadav SC, Yadav SK. Syzygium Cumini Leaf and Seed Extract Mediated Biosynthesis of Silver Nanoparticles and Their Characterization. J Chem Technol Biotechnol 2010; 85: 1301-09.

[17] Zhan G, Huang J, Du M, Abdul-Rauf I, Ma Y, *et al*. Green Synthesis of Au-Pd Bimetallic Nanoparticles: Single Step Bioreduction Method with Plant Extract. Mat Lett 2011; 65: 2989-91.

[18] Nadagouda MN, Varma RS. Green Synthesis of Silver and Palladium Nanoparticles at Room Temperature Using Coffee and Tea Extract. Green Chem 2008; 10: 859-62.

[19] Das RK, Borthakur BB, Bora U. Green Synthesis of Gold Nanoparticles Using Ethanolic Leaf Extract of Centella Asiatica. Mat Lett 2010; 64: 1445-47.

[20] Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of Silver Nanoparticles Using Citrus Sinensis Peel Extract and its Anti-bacterial activity. Spectrochimica Acta Part A: Mol Biomol Spectroscop 2011; 79: 594-98.

[21] Song JY, Kim BS. Biological Synthesis of Bimetallic Au/Ag Nanoparticles Using Persimmon (Diopyros kaki) Leaf Extract. Korean J Chem Eng 2008; 25: 808-11.

[22] Song JY, Kwon EY, Kim BS. Biological Synthesis of Platinum Nanoparticles Using Diopyros Kaki Leaf Extract. Bioprocess Biosys Eng 2010; 33: 159-64.

[23] Sathishkumar M, Sneha K, Kwak IS, Mao J, Tripathy SJ, *et al*. Phyto-Crystallization of Palladium Through Reduction Process Using Cinnamom Zeylanicum Bark Extract. J Hazardous Mat 2009; 171: 400-04.

[24] Yang X, Li Q, Wang H, Huang J, Lin L, *et al*. Green Synthesis of Palladium Nanoparticles Using Broth of Cinnamomum Camphora Leaf. J Nanopart Res 2010; 12: 1589-98.

[25] Bankar A, Joshi B, Kumar AR, Zinjarde S. Banana Peel Extract Mediated Novel Route for the Synthesis of Palladium Nanoparticles. Mat Lett 2010; 64: 1951-53.

[26] El-Abhar HS, Shaalan M, Barakat M, El-Denshary ES. Effect of melatonin and nifedipine on some antioxidant enzymes and different energy fuels in the blood and brain of global ischemic rats. J Pineal Res 2002; 33: 87-94.

[27] Garnett M. US Patent, US 776,973, 1998.

[28] Garnett M. US Patent, US 463,093, 1995.

[29] Lakshmy R, Krishnan CN. Therapeutic Potentials of Silver Nanoparticle Complex of α-Lipoic Acid, Nanomater Nanotechnol 2011; 1: 17-24.

[30] Kanchana A, Saveetha D, Rathakrishnan SA. Green synthesis and characterization of palladium nanoparticles and its conjugates from solanum trilobatum leaf extract. Nano-Micro Lett 2010; 2: 169-76.

[31] Lushchak VI, Semchyshyn HM, Lushchak OV. The Classic Methods to Measure Oxidative Damage: Lipid Peroxides, Thiobarbituric-Acid Reactive Substances, and Protein Carbonyls, in Oxidative Stress in Aquatic Ecosystems (eds Abele D, Vázquez-Medina JP, Zenteno-Savín T), Chichester, UK, John Wiley & Sons, Ltd: 2011.

[32] Sait C, Hatic A. Total Antioxidant Capacity, Catalase and Superoxide Dismutase on Rats Before and After Diabetes. J Animal Vet Adv 2009; 8: 1503-08

[33] Chuian-Fu K, Hsueh-Tai C, Reny-Chang C, Chi-Tsai L. Biochemical characterization of a catalase from Antrodia camphorata: Expression in Escherichia coli and enzyme properties. Bot Stud 2008; 49: 119-25.

[34] Kara H, Cevik A, Konar V, Dayangac A, Servi K. Effects of selenium with vitamin E and melatonin on cadmium-induced oxidative damage in rat liver and kidneys. Biol Trace Elem Res 2008; 125:236-44.

[35] Lowry OH, Roserberry NJ, Farr AI, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265-75.

[36] Karaman M, Akkemik E, Budak H, Ciftci M. In vitro effects of some drugs on human erythrocyte glutathione reductase. J Enzyme Inhib Med Chem 2012; 27:18-23.

[37] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Coll Interf Sci 2009; 145: 83-96.

[38] Shan B, Cai YZ, Sun M, Corke.H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem 2005; 53: 7749– 59.

[39] Monavalli B, Raja Rajeswari A, Gowri V, Kanchana A. *Invitro* Antioxidant Activity of Methanolic Extract of *Solanum Trilobatum* Leaves. J Natural Sci Technol Life Sci Bioinform 2010; 2: 168-74.

[40] Yonezawa T, Kawasaki H, Tarui A, Watanabe T, Arakawa R, *et al.* Detailed investigations on the possibility of nanoparticles of various metal elements for surface-assisted laser desorption/ionization mass spectrometry. Anal Science 2009; 25: 339-46.

[41] Basu S, Pal T. Glutathione-Induced Aggregation of Gold Nanoparticles: Electromagnetic Interactions in a Closely Packed Assembly. J Nanosci and Nanotechnol 2007; 7: 1904-10.

[42] Subramaniam C, Tom RT, Pradeep T. On the formation of protected gold nanoparticles from Aucl4- by the reduction using aromatic amine. J Nanopart Res 2005; 7: 209-17.

[43] Aslam M, Fu L, Su M, *et al*. Novel one step synthesis of amine stabilized aqueous colloidal gold nanoparticles. J Mat Chem 2004; 14: 1795-97.

[44] Mee-Kyung C, Schlaf M. A catalytic synthesis of thiosilanes and silthiane: palladium nanoparticle-mediated cross-coupling of silanes with thio phenyl and thio vinyl ethers through selective carbon-sulfur bond activation. J Am Chem Soc 2004;126: 7386-92.

[45] Chen A, Goldbreg M, Horwitz N, *et al.* Phys. Rev. Lett 1984; 52: 1084-88.

[46] Ruoslahti E. Targeting tumour vasculature with homing peptides from phage display. Seminars in Cancer Biol 2000; 10: 435-42.

[47] Kanchana A, Nina LM, Jayshree N, *et al*. Facile synthesis of biocompatible gold nanoparticles from Vites vinefera and its cellular internalization against HBL-100 cells. Cancer Nanotechnol 2011; 2: 121-3.

[48] Yong P, Rowson NA, Farr JPG, Harris IR, Macaskie LE. Bioreduction and biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307. Biotechnol Bioeng 2002; 80: 369-79.

[49] Mallikarjuna N, Nadagouda Rajender SV. Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chem 2008; 10: 859-62.

[50] Mahmoudi M, Simchi A, Milani AS, Stroeve P. Cell toxicity of superparamagnetic iron oxide nanoparticles. J Coll Interf Sci 2009; 336: 510-18.

[51] Garnett WA, Garnett M. Charge relay from molybdate oxyradicals to palladium-lipoic complex to DNA. Conference on Oxygen Intermediates in Non heme Metallobiochemistry Abstract. 1996; 81.

[52] Lin W, Huang YW, Zhou XD, Yinfa M. In vitro toxicity of silica nanoparticles in human lung cancer cells. Toxicol Appl Pharmacol 2006; 217: 252-59.

[53] Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, *et al*. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fiber Toxicol 2005; 2: 1-35.

[54] Sudheesh NP, Ajith TA, Janardhanan KK, Krishnan CV. Palladium alpha-lipoic acid complex formulation enhances activities of Krebs cycle dehydrogenases and respiratory complexes I–IV in the heart of aged rats. Food Chem Toxicol 2009; 47: 2124-28.

[55] Antonawich FJ, Fiore SM, Welicky LM. Regulation of ischemic cell death by the lipoic acid palladium complex, Poly-MVA, in gerbils. Exp Neurol 2004; 189: 10-15.

[56] Udo S, David H, Marilena PR, *et al*. Helix pomatia agglutinin binding is a useful prognostic indicator in colorectal carcinoma. Cancer 1994; 74: 3104-07.

[57] Shvedova AA, Castranova V, Kisin ER, Schwegler-Berry D, Murray AR *et al*. Exposure to Carbon Nanotube Material: Assessment of Nanotube Cytotoxicity using Human Keratinocyte Cells. J Toxicol Env Health Part A 2003; 66: 1909-26.

[58] Gurr JR, Alexander SSW, Chien HC, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicol 2005; 15: 66-73.

[59] Halliwell B, Gutteridge JM. Biochemistry of oxidative stress. Free Rad Biol Med 1995; 18: 125-26.

[60] Trachootham D, Lu W, Ogasawara MA, Nilsa RD, Huang P. Redox Regulation of Cell Survival. Antioxid redox signal 2008; 10: 1344-65.

[61] Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Rad Biol Med 1990; 8: 583-99.

[62] Shahjahan M, Vani G, Shyamaladevi CS. Effect of *Solanum trilobatum* on the antioxidant status during diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat. Chem Biol Interact 2005; 156: 113-23.

[63] Mohanan PV, Devi KS. Cytotoxic potential of the preparations from *Solanum trilobatum* and the effect of sobatum on tumour reduction in mice. Cancer Lett 1996; 17: 71-76.

[64] Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. Biochim Biophys Acta - General Subjects 2009; 1790: 1149-60.

[65] Packer L, Cadenas E. Lipoic acid: energy metabolism and redox regulation of transcription and cell signalling. J Clin Biochem Nutr 2011; 48: 26–32.

[66] Packer L, Witt EH, Tritschler HJ. Alpha-Lipoic acid as a biological antioxidant. Free Radic Biol Med 1995;19:227-50.

[67] Rudich A, Tirosh A, Potashnik R, *et al*. Lipoic acid protectsagainst oxidative stress induced impairment in insulin stimulation of protein kinase B and glucose transport in 3T3-L1 adipocytes. Diabetologia 1999; 42:949

[68] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother 2003;57: 145–55

[69] Sudheesh NP, Ajith TA., Janardhanan K.K., Krishnan CV. Effect of POLYMVA, a palladium α-lipoic acid complex formulation against declined mitochondrial antioxidant status in the myocardium of aged rats. Food ChemToxicol 2012; 48: 1858-62.

[70] Sudheesh NP, Ajith TA, Janardhanan K.K, Krishnan CV. Palladium-α-lipoic acid complex attenuates alloxan-induced hyperglycemia and enhances the declined blood antioxidant status in diabetic rats. J Diabetes 2011; 3: 293-300

[71] Moini H, Packer L, Saris NEL. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol 2002; 182:84-90.

[72] Simbula G, Columbano A, Ledda-Columbano GM, Sanna L, Deidda M, *et al*. Increased ROS generation and p53 activation in alpha-lipoic acid-induced apoptosis of hepatoma cells. Apoptosis 2007; 12: 113–23.

[73] Moungjaroen J, Nimmannit U, Callery PS, Wang L, Azad N, *et al*. Reactive oxygen species mediate caspase activation and apoptosis induced by lipoic acid in human lung epithelial cancer cells through Bcl-2 down-regulation. J Pharmacol Exp Ther 2006; 319: 1062–69.

[74] Zheng Y, Yamaguchi H, Tian C, Lee MW, Tang H, *et al*. Arsenic trioxide (As(2)O(3)) induces apoptosis through activation of Bax in hematopoietic cells. Oncogene 2005; 24: 3339–47.

[75] Singh LP, Cheng DW, Kowluru R, Levi E, Jiang Y. Hexosamine induction of oxidative stress, hypertrophy and laminin expression in renal mesangial cells: Effect of the anti-oxidant alpha-lipoic acid. Cell Biochem Funct 2007; 25: 537–50.

[76] Abdul HM, Butterfield DA. Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. Free Radic Biol. Med. 2007, 42, 371–84.

[77] Choi SY, Yu JH, Kim H. Mechanism of alpha-lipoic acid-induced apoptosis of lung cancer cells. Ann NY Acad Sci 2009; 1171: 149–155.

[78] Gao C, Wang AY. Significance of Increased Apoptosis and Bax Expression in Human Small Intestinal Adenocarcinoma. J Histochem Cytochem 2009; 57: 1139-48.

[79] Wenzel U, Nickel A, Daniel H. α-Lipoic acid induces apoptosis in human colon cancer cells by increasing mitochondrial respiration with a concomitant O_{22} generation. Apoptosis 2005; 10: 359-68.