



Lantana aculeata L.-Mediated Zinc Oxide Nanoparticle-Induced DNA Damage in *Sesamum indicum* and Their Cytotoxic Activity Against SiHa Cell Line

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15.1 Introduction

In recent years, advanced science and technology researchers have attempted to synthesize nanoparticles (NPs) within the size range of 1–100 nm, and this extensive research and concern on NPs is enlarging because of their wide application in areas of science and technology. Zinc oxide NPs belong to the class of metal oxides, which is characterized by photocatalytic capacity against chemical and biological species (Srivastava 2007). The progress of technology and life quality of mankind has been closely with the progress in material science. Most techniques applied in material processing are based on breaking up large mass of a material into preferred sizes and shapes in the processed material (Roco et al. 1999). Late improvements depend on the impact of different quantum size nanoscale particles, uncovering that the greater part of the novel work will be founded on properties of nanomaterials. The traditional processing techniques that provoke lattice defects and further imperfections will no longer be thinned for synthesis of nanoparticle by absolute number of atoms (Isobe et al. 2006). Moreover, the purposes of traditional draw near impart difficulties for synthesis of such small particles in an enviable size range.

Alternative artificial technique for NPs involves proscribed precipitation of NPs from precursors and dissolved in a solution (Warheit 2008). A micro suspension can also be formed using surfactants between two immiscible liquids, with the intransigent isolated inside a colloid, through hydrophobic in opposition to hydrophilic

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forces (Masciangioli and Zhang 2003). The resultant NPs form a micro-colloidal suspension. The mechanism concerned in stabilization of NPs can be categorized as (Smith et al. 2007) electrostatic stabilization, concerning the creation of a double layer of adsorbed ions over the NPs resulting in a coulombic repulsion between approaching NPs, or steric hindrance, achieved by adsorption of polymer molecules over the NPs.

Nature has devised processes for the synthesis of nanoscaled inorganic materials which have contributed to the improvement of moderately new and largely uncultivated area of research based on the biosynthesis of nanomaterials (Papis et al. 2007). Synthesis using plant extract is congruent with the green chemistry principles. "Green synthesis" of NPs makes use of environmental friendly, harmless reagents and non-toxic.

15.1.1 Nanoparticle Concept and Production

Nanoparticles belong to the wider group of nanomaterial, where the prefix "nano" refers to infinitesimal physical dimension. Hence nanoparticles possess properties that are qualitatively or quantitatively distinctly different from their other physical forms (SCENIHR 2006), such as those of large size particles (bulk particles) made from the same material and their water-soluble form. Size-related differences in particle properties may be due to the large surface area per mass, resulting in increased ratio of surface to core atoms and increased number of corner and edge atoms. This results in increased reactivity or increased ion released (Elzey and Grassian 2010), which enables their use in novel applications.

Theoretically, engineered nanoparticles (ENPs) can be created from any substance; however normally the vast majority of the ENPs are integrated from transition metals, silicon, single-walled carbon nanotubes, fullerenes and metal oxide. Top-down and bottom-up creation are two specific methodologies for the era of nanoparticles. In top-down strategy, lithographic systems are utilized to cut vast bits of a material into NPs (Powell et al. 2008). The worldwide business sector for nanotechnology was esteemed at about \$20.1 billion in 2011 and should reach \$20.7 billion in 2012, and the production will probably increase sharply in the near future.

15.1.2 Plants as a Source of Nanomaterial Synthesis

Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticle formation. It consolidates natural standards with physical and compound methods to create nanosized particles with particular capacities. Although chemical and physical methods may effectively create produce pure, well-defined nanoparticles, these strategies are very costly and possibly perilous to nature (Chandran et al. 2006). The preparation of nanoparticles utilizing green technologies is favourable over substance specialists because of their ecological

results. The biological method of synthesis of nanoparticles has proved to be a better method than other chemical methods due to the large amount of capital involved in the production of energy-intensive processes (Mukherjee et al. 2014).

Gardea-Torresdey et al. (2002) have demonstrated that gold and silver nanoparticles are formed within different parts of live alfalfa plant on accumulating the corresponding metal ions from solid media. In an attempt towards deliberate synthesis of metal nanoparticles, plant extracts like that of neem (*Azadirachta indica*) (Shankar et al. 2004), geranium (Shankar et al. 2003) and amla (*Emblia officinalis*) (Ankamwar et al. 2005) can be used for the size- and shape-directed biosynthesis of gold, silver (Gardea-Torresdey et al. 2002) and gold and silver bimetallic core-shell nanoparticles (Shankar et al. 2004).

Utilization of natural living beings, for example, microorganisms, plant extract or plant biomass, could be a distinct option for synthetic and physical methods for the generation of nanoparticles in an eco-accommodating way (Elumalai et al. 2010). The most important application of silver and silver NPs is in restorative industry such as topical ointments to prevent infection against burn and open injuries (Ismail and Bakar 2004).

15.1.3 Description of Plants

15.1.3.1 General Description of *Lantana aculeata*

The word *Lantana aculeata* is derived from Latin 'lento' which means to bend. The species was initially portrayed and given its binomial name by Linnaeus in 1753 (Kumarasamyraja et al. 2012). It is a member of the Verbenaceae family with 600 assortments existing around the world. *Lantana aculeata*, a native species of South and Central America and the Caribbean islands, has its presence recorded even in Brazil, Florida, Jamaica, Mexico and Trinidad. The species is spread over to a wide geological extent in neotropics, yet none is accounted for from the Old World (Day et al. 2003). Some species of *Lantana aculeata* (Fig. 15.1) are also believed to originate from Africa and one from India (Hiremath and Sundaram 2005).


Kingdom	: Plantae	
Order	: Lamiales	
Family	: Verbenaceae	
Genus	: <i>Lantana</i>	
Species	: <i>L. aculeate</i>	

Fig. 15.1 Taxonomy of *Lantana aculeata*

Biology of *Lantana aculeata*

Lantana aculeata, also known as wild sage, is a thorny multi-stemmed, deciduous shrub with an average height of 2 m. The shrub's taxonomic position is characterized as fitting in with class Magnoliopsida, order Lamiales, family Verbenaceae and genus *Lantana* (Larson et al. 2001). Stems are square in blueprint, secured with bristly hairs when green, regularly furnished or with scattered little prickles. *Lantana aculeata* possesses a strong root system (Kumarasamyraja et al. 2012). The roots even after rehashed cuttings give new flush of shoots. Leaves are inverse, basic, with long petioles, oval cutting edges which are unpleasant, bristly and have gruff toothed edges. The leaves of *Lantana aculeata* have a solid smell. Its blossoms are little, multihued, in stalked, thick in level finished bunches with a corolla having slender tube with four short spreading projections. Their blossoms experience shading change consequent to anthesis. These blossoms happen in bunch which incorporates white-pink-lavender or yellow-orange-red blend (Hiremath and Sundaram 2005). The yellow shading of the blossom gives visual signal to pollinators, and change in shading is started on the demonstration of fertilization. Berries of *Lantana aculeata* are round, plump, two-seeded drupe with at first green in shading and turning purple lastly to blue-dark shading (Day et al. 2003). Be that as it may, the berries are extremely toxic in nature; however these are alluring to creepy crawlies and winged creatures. Seed germination is simple and speedier in *Lantana aculeata*.

Uses

Lantana aculeata though being a noxious weed has several minor uses, mainly in herbal medicine. There are series of research studies conducted on the exploitation of chemical constituents present in different parts of the plant species. The studies demonstrate that extracts from the leaves can be employed to combat antimicrobial, fungicidal, insecticidal and nematicidal problems. Its potential to serve as biocide has also been illustrated in several researches (Dobhal et al. 2011).

Impacts

Lantana aculeata has many negative impacts including potential to disrupt succession cycle, displacing native biota resulting in decreased biodiversity. Its infestations alter the structural and floral composition of native communities (Sharma and Raghubanshi 2010). As the density of *Lantana aculeata* in forest increases, allelopathic interactions increase, and hence there is decline in species richness (Day et al. 2003).

Lantana aculeata is a noteworthy issue in agricultural areas in most regions of India as it forms dense thickets, spreads gregariously, outcompetes pasture species and affects both flora and fauna. The field cases happen mostly in youthful creatures that have either been recently brought into a range where *Lantana aculeata* develops or are without access to other grubs. Children and adults in many countries often consume ripe fruits of *Lantana aculeata* without any ill effects. However, consumption of green fruit has proved to be fatal in some parts of India (Sharma et al. 2007). Apart from causing death of livestock, sublethal doses of *Lantana aculeata* toxin

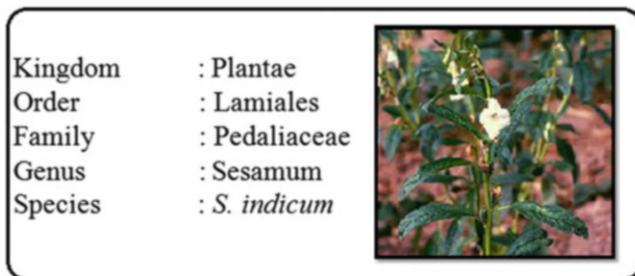


Fig. 15.2 Taxonomy of *Sesamum indicum*

cause reduction in potential production, manifested abortion, loss of milk production in dairy cows and chronic wasting in beef cattle.

15.1.3.2 *Sesamum indicum*

Sesamum is a flowering plant (Fig. 15.2). The wild variety of *Sesamum* occurs in Africa and small number in India. It is widely established in tropical region around the world and is cultivated mainly for its edible seeds, which grow in pods. *Sesamum* seed is one of the oldest seed crops, domesticated well over 3000 years ago. It is originated in India. The world's largest exporter of *Sesamum* seed is India, and the largest importer is Japan. Its annual plant grows in 50–100 cm (1.6–3.3 ft) tall, with opposite leaf 4 ft and 14 cm (1.6–5.5 in.) long with an enter margin; there are broad lanceolate, to 5 cm (2 in.) broad at the base of the plant.

Description of *Sesamum indicum*

Sesamum indicum is an annual broadleaf plant that grows 5–6 ft tall. It produces a 1–2-in.-long white, bell-shaped inflorescence growing from the leaf axils (where the leaf stalk joins the stem). The blooms do not open all at once, but gradually, from the base of the stem upwards to the top of the plant. The flowers are both male and female and will self-pollinate (Monalisa and Patra 2013). The seed is produced in a 1–1.5-in.-long, divided seed capsule that opens when the seeds are mature. There are eight rows of seed within each seed capsule, and seed may be yellow, white, brown or black. Due to the non-uniform, indeterminate nature of the bloom period, the reproductive, ripening and drying phases of the seed tend to overlap. Seed lowest on the plant will mature first, even as the upper part of the plant is still flowering or has just formed seed capsules.

Cultivation

Sesame is very drought-tolerant, in part due to its extensive root system. However, it requires adequate moisture for germination and early growth. While the crop survives drought, as well as presence of excess water, the yields are significantly lower in either condition. Moisture levels before planting and flowering impact yield most (Purakayastha and Bhatnagar 1997).

Most commercial cultivars of sesame are intolerant of waterlogging. Rainfall late in the season prolongs growth and increases high harvest-shattering losses. Wind can also cause shattering at harvest. Initiation of flowering is sensitive to photoperiod and to sesame variety (Rahman et al. 2013). The photoperiod also impacts the oil content in sesame seed; increased photoperiod increases oil content. The oil content of the seed is inversely proportional to its protein content. Sesame varieties have adapted too many soil types. The high-yielding crops thrive best on well-drained, fertile soils of medium texture and neutral pH. However, these have low tolerance for soils with high salt and waterlogged conditions. Commercial sesame crops require 90–120 frost-free days. Warm conditions above 23 °C (73 °F) favour growth and yields. While sesame crops can grow in poor soils, the best yields come from properly fertilized farms (Ismail 2012; Selvi and Gunaseeli 2004).

15.1.4 Green Synthesis of Nanoparticles from *Lantana aculeata*

Lantana aculeata Linn, family Verbenaceae, is a shrub available throughout central and south India. It is currently the major outlandish weed, spreading quickly in wastelands and rural fields (Raghubanshi and Tripathi 2009). This plant can be used for the synthesis of nanoparticles in eco-friendly manner because it has the capacity to extract heavy metals through its roots, stems and leaves and also due to its rapid propagation (Zhang et al. 2002).

Thirumurugan et al. (2011) demonstrate the AgNP synthesis using leaf extract of *Lantana aculeata*. The silver nanoparticles synthesized were distinguished using scanning electron microscopy (SEM) which showed the approximate size of nanoparticles about 39.60 nm. Kumarasamyraja and Jaganathan (2013) reported that the silver nanoparticles were synthesized using the aqueous extract of *Lantana aculeata* and assessed their antimicrobial activity. The synthesized silver nanoparticles were characterized by UV-visible spectrophotometer. The size and shape of silver nanoparticles was confirmed by particle size analyses and TEM. The particle size ranged 0.772 nm. *Lantana aculeata*-mediated silver NPs showed better antimicrobial activity.

15.1.5 Zinc Oxide Nanoparticles

Zinc oxide is an inorganic compound of white powder generally insoluble in water. Mechanical properties such as internal stress or adhesion are important in order to assure the patterning accuracy and durability for various types of commercial applications. The structure of zinc oxide is generally hexagonal wurtzite, spherical and zinc blende (Krishnan and Pradeep 2009).

15.1.5.1 Synthesis and Characterization of Zinc Oxide Nanoparticles

There are numerous physical and chemical methods for synthesis of zinc oxide nanoparticle in huge quantities in a short period of time. Simple solution-based

methods, chemical precipitation, sol gel, solvothermal, electrochemical and photochemical reduction methods, are the most preferable methods. Zinc oxide NPs can also be synthesized from plant, microorganisms (bacteria and fungi) and enzymes by using green amalgamation techniques. Green synthesis methods are eco-accommodating approach and perfect for pharmaceutical and other biomedical applications furthermore in horticulture in light of the fact that no poisonous chemicals are utilized as a part of these strategies.

Nanoparticles have increased expanding consideration on account of their novel properties, including an extensive particular surface region and high response activity (Babu and Narayanan 2013). Nanoparticles are atomic or molecular aggregates with no less than one measurement between 1 and 100 nm that can definitely change their physiological properties contrasted with the mass material (Nel et al. 2006; Roco 2003). The synthesis of nanoparticles by routine physical and chemical techniques has some unfavourable impacts like basic states of temperature and weight, costly and poisonous chemicals, long reflux time of response and harmful side effects (Vanaja et al. 2013; Iravani 2011). When compared to physical and chemical method, green synthesis of nanoparticles makes utilization of ecological cordial, non-dangerous and safe reagents (Mohanpuria and Yadav 2009). The effect of temperature on nanoparticle formation also has been investigated, and it has been reported that polydisperse particles with a size of 5–300 nm were obtained at lower temperature, while a higher temperature supported the formation of much smaller and spherical particles (Song and Kim 2009).

ZnO nanoparticles have been synthesized using the plant extracts of *Eichhornia crassipes*-mediated ZnO nanoparticles were biosynthesized and its effect were seen against antifungal activity (Vanathi et al. 2014). Vidhya et al. (2013) have portrayed *Calotropis gigantea*-mediated ZnO NPs. The particles obtained were spherical in shape and were agglomerates of nanocrystallites. The average crystallite size estimated from XRD analysis was in the range of 30–35 nm. ZnO NPs were green synthesized by Sangeetha et al. (2011) using aloe leaf broth extract. Their outcomes showed improved biocidal activity against different pathogens when compared to chemically synthesized ZnO NPs. They have also reported that the effectiveness of NPs increased with increased particle dose, treatment time and synthesis method. Nagarajan and Kuppasamy (2013) have reported the green route biosynthesis of ZnO NPs and their utility as catalyst. The nanoparticles are characterized by UV-Vis, FTIR, XRD, TGA, SEM-EDX TEM and GC-MS techniques. The obtained particles were in size range of 8–32 nm and also very stable even after a month.

15.1.5.2 Application of Zinc Oxide Nanoparticles

Once particles are synthesized, they significantly change their physical and chemical properties. The typical properties of the particle like heat treatment, mass exchange, synergist movement, etc. all change, but compared to non-metal nanoparticles, metal nanoparticles have more industrial applications. Nanoparticles offer much new development in the field of biosensors, biomedicine and bio-nanotechnology specifically in the areas such as drug delivery, medical diagnostic tools, cancer treatment agent (gold nanoparticles) and agriculture as bio-fertilizers to plants. Magnetic

nanoparticles are getting significant consideration due to their extensive variety of utilizations, such as the immobilization of the proteins and enzymes, bio-separation, immunoassays, drug delivery and biosensors. Nanoparticles and nanostructure are becoming a part in human medical applications, including imaging or the delivery of therapeutic drugs to cell, tissues and organs (Harter and Naidu 2001).

It is an important conventional band gap semiconductor with tremendous scientific and technological interest, having a direct wide gap (3.37 eV) (Huang et al. 2001). It is an exceedingly favoured multi-tasking metal oxide having an immeasurable rundown of appealing properties and has been generally utilized as a part of numerous modern ranges, for example, sun-oriented cells, UV-light-radiating gadget, gas sensor, photocatalysts, pharmaceutical and restorative commercial enterprises (Yang and Park 2008). It is non-harmful, self-purging (Yadav et al. 2006), perfect with skin, antimicrobial, dermatological, utilized as an UV blocker as a part of sunscreen and numerous biomedical applications (Krishnan and Pradeep 2009). The benefits of ZnO is bio-protected, biocompatible with extraordinary capacity like structure ward properties, electrical and warm transport properties, which could be changed as for molecule size, shape, morphology, introduction and perspective proportion, have brought about expanded enthusiasm for acquired this nano-metal oxide material (Dakhlaoui et al. 2009).

15.1.6 Cytotoxicity of Zinc Oxide Nanoparticles

ZnO nanoparticles show relatively high biocompatibility. Their bulkier form is generally recognized as safe (GRAS) by the FDA. Zinc is an important cofactor in various cellular mechanisms and plays an important role in maintaining cellular homeostasis; hence ZnO shows biocompatibility. The administered ZnO can be easily biodegraded or can take part in the active nutritional cycle of the body (Choudhury and Panda 2004). While extracellular ZnO shows biocompatibility, elevated levels of administered intracellular ZnO show enhanced cytotoxicity through zinc-mediated protein activity disequilibrium and oxidative stress (Kahru and Dubourguier 2010). ZnO nanoparticles have the unique ability to induce oxidative stress in cancer cells, which has been found to be one of the mechanisms of cytotoxicity of ZnO nanoparticles towards cancer cells. This property is due to the semiconductor nature of ZnO. ZnO induces ROS generation, leading to oxidative stress and eventually cell death when the anti-oxidative capacity of the cell is exceeded.

15.1.6.1 Mechanism of Cytotoxicity

The basic mechanism behind the cytotoxicity of ZnO NPs is the intracellular release of dissolved zinc ions, followed by ROS induction. This event causes zinc-mediated protein activity disequilibrium and oxidative stress, eventually killing the cell. Soluble extracellular zinc shows very little cytotoxicity. Recent research shows that extracellular soluble zinc, when exposed to cell culture and media, forms poorly soluble amorphous zinc-carbonate phosphate precipitates (phosphate from media).

This precipitate is supposed to protect the cell from the cytotoxicity of zinc (Kasemets et al. 2009). On the other hand, with the release of soluble zinc ions inside the cell, a cascade of pathways interrelated to each other takes place, which is responsible for the cytotoxic response of the ZnO nanoparticles.

Many in vitro studies have proved that ZnO NPs show selective cytotoxicity towards cancer cells. Jiang suggested that they show 28–35 times selective toxicity towards cancer cells compared with that of normal cells (Jiang et al. 1998). This selective cytotoxicity in cancer cells in in vitro condition can also be further exploited in the in vivo condition by selectively targeting ZnO nanoparticles towards cancer cells. ZnO NPs selectively kill cancer cells by inferring selective localization and selective cytotoxicity towards them.

15.1.7 Genotoxicity of Zinc Oxide Nanoparticles

DNA-damaging potential of ZnO nanoparticles in *Sesamum indicum* as representative of plant system could be confirmed in the comet assay and DNA laddering experiment. Comet test likewise called single-cell gel electrophoresis is a system for the location of DNA harm at the level of individual cells, which is a standout amongst the most exceptional methods acquainted with the agricultural sciences as of late. The assay test is the most popular tests of DNA damage detection by electrophoresis (Chakraborty et al. 2009). The assay is quick, simple to handle, non-obtrusive, visually and reasonably contrasted with most traditional procedure. Thus, it has rapidly gained importance in the field of genetic medicine, toxicology, agriculture and environmental studies (Srivastava 2007). The fundamental guideline of this test is to decide the DNA break by measuring the DNA harm which is evaluated by the extent of DNA, which moves out of the cores towards the anode when singular cell or isolated nuclei are embedded in a thin agarose layer. Diameter of nuclei of the studied species and the degree of DNA denaturation indicate the condition of DNA and are responsible for metabolic activities.

In previous study, a simple, rapid biological procedure has been evolved to synthesize ZnO nanoparticles from *Lantana aculeata* leaf broth extracted using Zn (NO₃) as precursor. The synthesized nanoparticles have been characterized by various techniques which include UV-Vis, FTIR, XRD, EDX, FESEM and HRTEM (Figs. 15.3, 15.4, 15.5, 15.6, 15.7 and 15.8). The biological synthesis of ZnO nanoparticles was spherical in shape with an average size of 12–25 nm. These study indicate the benefits of using biological method in synthesizing ZnO nanoparticles that have antimicrobial activities, and also it could be effective in agricultural development (Narendhran et al. 2016). In the present study is the continuation to assess the genotoxicity and cytotoxicity activities of *Lantana aculeata*-mediated zinc oxide nanoparticles.

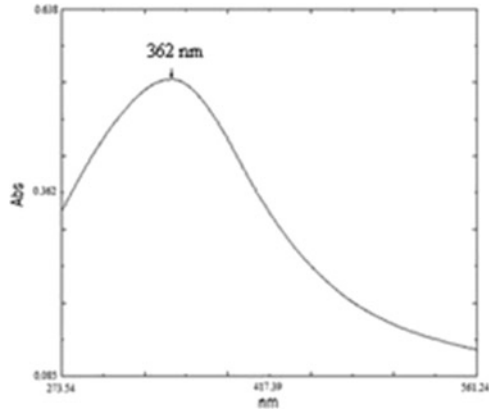


Fig. 15.3 UV spectrum of ZnO NPs

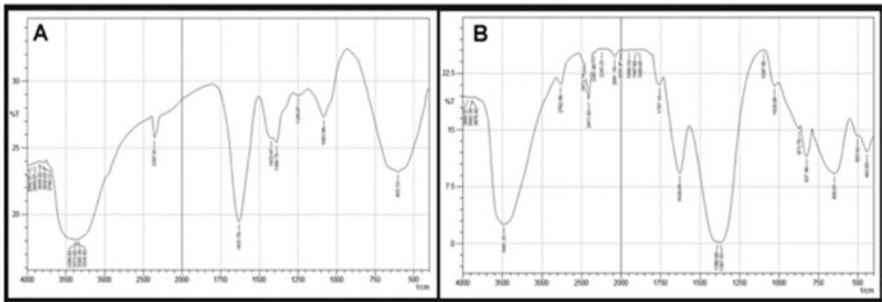


Fig. 15.4 FTIR spectrum of (a) *L. aculeata* leaf extract (b) *L. aculeata*-mediated ZnO nanoparticles

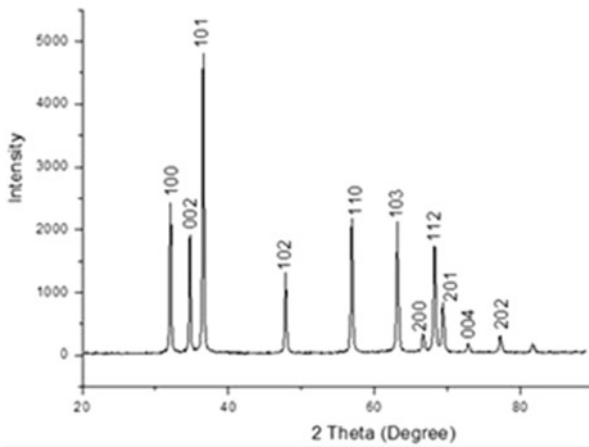


Fig. 15.5 XRD spectrum of ZnO nanoparticles

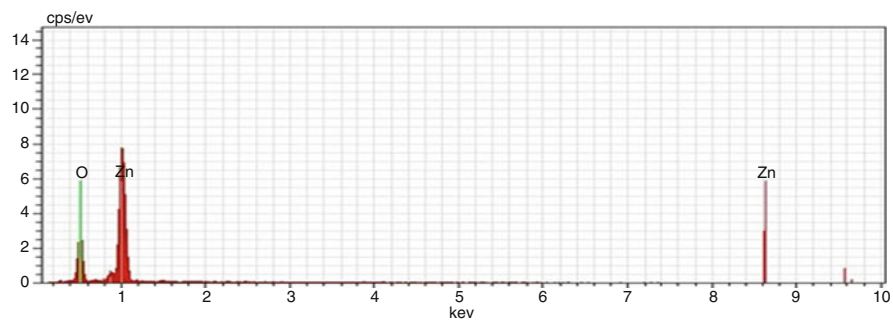


Fig. 15.6 EDX spectrum of *L. aculeata*-mediated ZnO nanoparticles

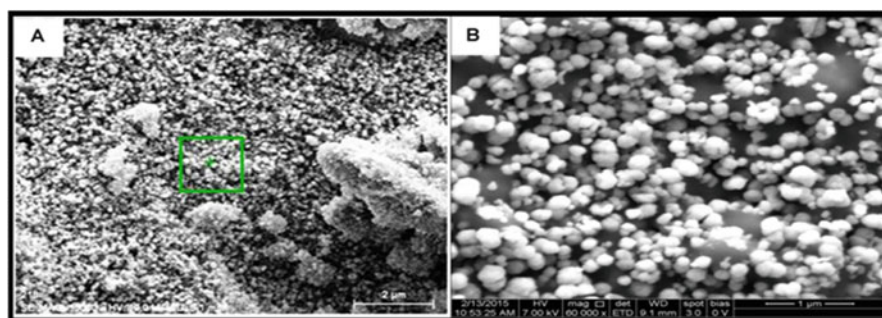


Fig. 15.7 (a and b) FESEM images of *L. aculeata*-mediated ZnO nanoparticles

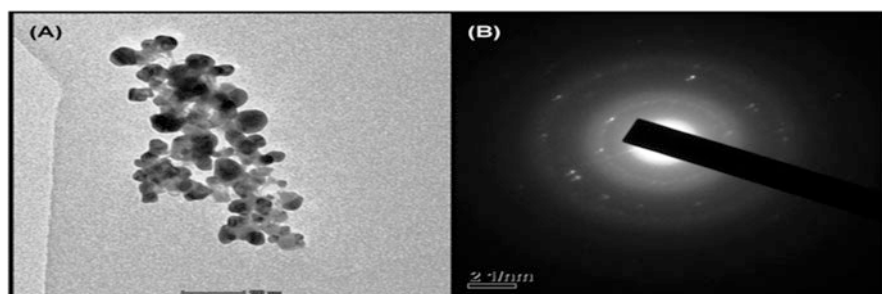


Fig. 15.8 (a) HRTEM images of *L. aculeata*-mediated ZnO nanoparticles (b) SAED pattern analysis of ZnO nanoparticles

15.2 Material

Fresh, healthy and young *L. aculeata* leaves were collected from Vadavalli region (11.0100° N, 76.9000° E), Coimbatore, Tamil Nadu, India. The sample was authenticated by Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2014-15/Tech/1418). *L. aculeata*-mediated ZnO nanoparticles synthesis with a particle size of 12–25 nm.

Zinc nanoparticles have been suspended using Milli-Q water and dispersed by ultrasonic vibration. For the present study, five concentrations, viz. 100, 250, 500, 1000 and 2000 mg L⁻¹, of ZnO NPs were used. *Sesamum indicum* (CO-1) seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min sterilization and experimental consistency following Narendhran et al. (2016). After rinsing three instances with Milli-Q water, sesame has been soaked in ZnO suspensions at soaking duration of 1 day. Milli-Q water was used in the soaking method for a better control of the media. A pot experiment was conducted at Karpagam Academy of Higher Education Campus, Eachanari, Coimbatore, Tamil Nadu, India, during July 2015. Ten seeds had been sown in each pot (30 cm diameter and 25 cm deep) on zinc-deficient soil. After 60 DAS, zinc oxide nanoparticles treated and untreated leaf samples of *Sesamum indicum* were collected in brown paper covers and brought to the laboratory. Leaves were washed with tap water and air-dried. The leaves were sealed in plastic sacks, marked and stored at 4 °C for further studies.

15.3 Methods

15.3.1 DNA Damage Analysis Using Comet Assay in *Sesamum indicum* (Chakraborty et al. 2009)

The *Sesamum indicum* leaves were put for 2 min on ice to keep them turgid. For isolation of nuclei, leaf tissues were put in a petric plate containing Tris buffer (400 mM, pH 7.5). Using a fresh razor blade, leaves were finely and gently sliced allowing isolation of nuclei. The segregated nuclei were gathered in the buffer. Taking the nuclear suspension, slides were put in alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA; pH > 13) for 15 min to permit loosening up of the DNA in a horizontal gel tank took after by electrophoresis at 4 °C for 20 min at 26 V adjusted to 300 mA by changing support level in the tank. Slides were kept in 0.4 M Tris (pH 7.5) for 5 min lastly rinsed in water. Every trial was rehashed twice.

15.3.2 DNA Extraction and Laddering

DNA was isolated from leaf of *Sesamum indicum* using a modified CTAB method (Khan et al. 2007). The leaves were weighed and ground in extraction buffer (25 mM EDTA, 100 mM Tris buffer pH 8, 3% PVP, 2 M NaCl, 3% CTAB). The suspension was gently mixed and incubated at 65 °C for 20 min with infrequent blending. It is used converted to room temperature and an equivalent volume of chloroform: isoamyl alcohol (24:1) was included. The blend was rotated at 12,000 rpm for

5 min. The reasonable upper fluid stage after that exchanged to another tube, to which 2/3 volume ice-cold isopropanol was added and incubated at 20 °C for 30 min. The experimental resulting pellet was washed twice with the 75% ethanol. After that the pellet needs to be air-dried under a clean laminar hood and then the nuclei acid dissolved in TE (10 mM Tris buffer pH 8.1 and 1 mM EDTA) at room temperature itself and then kept stored at 4 °C until before start to use. RNA is eliminated by treating the sample with RNase (10 mg or ml) for 30 min at 37 °C. The DNA purity is determined through measuring the absorbance of diluted DNA solution at 260 and 280 nm. The isolated DNA from all the treated samples was determined on 2.5% agarose gel in TAE (Tris acetate EDTA) buffer at 100 V, at 4 °C. 100 bp ladder was loaded for proper reference. DNA was stained with aqueous solution of EtBr, photographed and visualized under a UV transilluminator.

15.3.3 Determination of In Vitro Antiproliferative Effect of *Lantana aculeata*-Mediated Zinc Oxide Nanoparticles on Cultured SiHa Cell Lines

SiHa cervical cancer cell lines that were purchased from NCCS Pune were maintained in Dulbecco's Modified Eagle Media (HiMedia) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37 °C in 5% CO₂ in a humidified atmosphere in a CO₂ incubator (NBS, Eppendorf, Germany). The cells were trypsinized [500 µl of 0.025% trypsin in PBS/0.5 mM EDTA solution (HiMedia)] for 2 min and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25, 12.5, 25, 50 and 100 µg mL⁻¹ from a stock of 1 mg mL⁻¹ and incubated for 24 h.

The % difference in viability was determined by standard MTT assay after 24 h of incubation. MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent dimethyl sulfoxide (HiMedia), and the released, solubilized formazan product was measured at 540 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The cells were washed with 1x PBS and then 30 µl of MTT solution added to the culture (MTT –5 mg mL⁻¹ dissolved in PBS). It was then incubated at 37 °C for 3 h. MTT was removed by washing with 1x PBS, and 200 µL of DMSO was added to the culture. Incubation was done at room temperature for 30 min until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a microplate reader (ELISASCAN, ERBA). The % viability was determined using following formula:

$$\% \text{Viability} = (\text{OD of Test} / \text{OD of Control}) \times 100$$

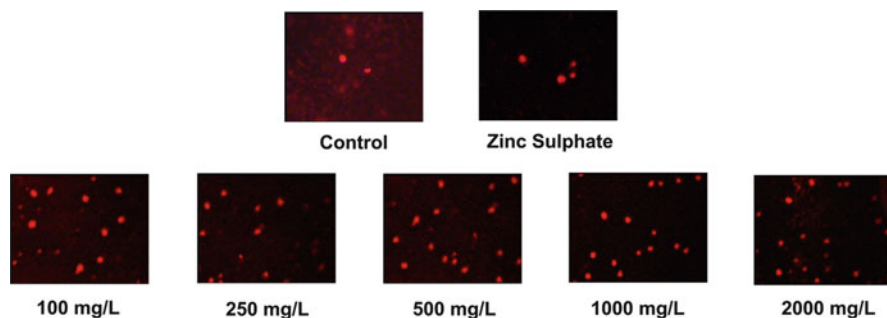


Fig. 15.9 Comet image of *Lantana aculeata*-mediated ZnO nanoparticle-treated *Sesamum indicum* leaf at different concentrations (mg L^{-1})

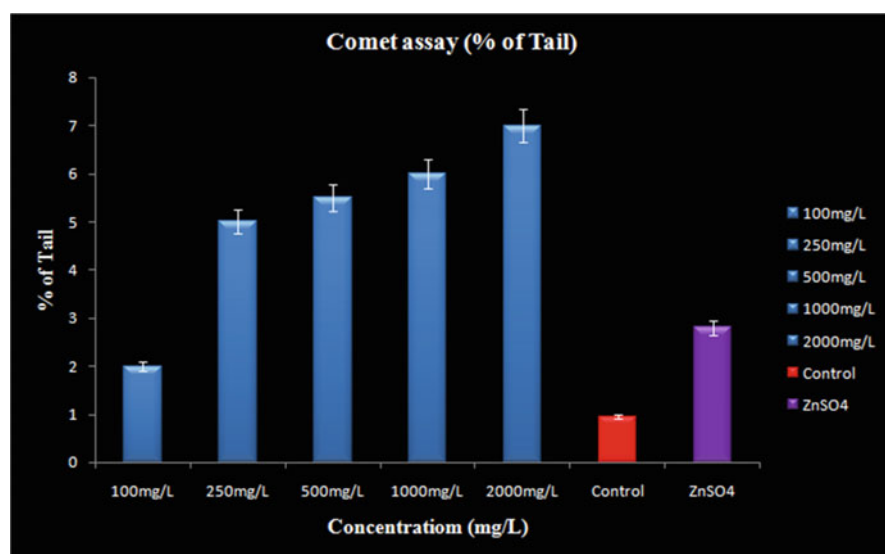


Fig 15.10 Graphical representation of ZnO nanoparticles (% tail DNA) at different concentrations

15.4 Result and Discussion

15.4.1 Comet Assay

The percentage (%) of the tail DNA in *Sesamum indicum* treated with *Lantana aculeata*-mediated ZnO is shown in Figs. 15.9 and 15.10. ZnO nanoparticles showed a sign of significant DNA damage at higher concentration and induced a dose-dependent increase in extent of DNA damage significantly at concentration above 1000 mg L^{-1} . It could be credited to a property of nanomaterials to frame agglomerates by goodness of which, with expansion in treatment focus the

nanoparticles tend to precipitate. The more prominent association of nanoparticles amongst themselves that could have expanded inferable from expansion in treatment fixation may have constrained the ZnO nanoparticles from communicating with the plant system (Zhang et al. 2005). It is evidenced that ZnO nanoparticles create large amount of hydroxyl free radical, thereby leading to DNA damage (Reeves et al. 2007). Abiotic stress (including heavy metal) results in DNA injury or damage to plant cell also straight or not directly (Kumari et al. 2009). Atha et al. (2012) reported that CuO NPs induced DNA harm or damage in agricultural and grassland plants. However, improved antioxidant enzyme (POD, SOD and CAT) activity in plant root tissues exposed to CuO and ZnO nanoparticle was observed.

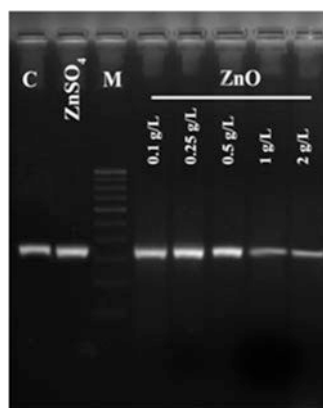
15.4.2 Isolation of DNA

The DNA that was isolated from phytomediated ZnO nanoparticles in *Sesamum indicum* was further evaluated by DNA laddering. The outcome can be in all likelihood be related by means of that got as of comet test. While the negative control set indicated nearness of unharmed genomic DNA represented to by a thick band on the agarose gel, the most astounding degree of DNA harm was seen at ZnO nanoparticle treatment. The gel (Fig. 15.11) also clearly indicated an initial increase in DNA damage up to 1000 mg L⁻¹ followed by subsequent decrease in extent of DNA injury/damage along with growing treatment attentions.

15.4.3 Cytotoxicity Study

The cytotoxicity of the zinc oxide nanoparticles was evaluated against SiHa cervical cancer cell lines at various concentrations (6.5–100 µg mL⁻¹). Figures 15.12 and 15.13 show the cytotoxic activity of zinc oxide nanoparticles, and IC₅₀ value for zinc oxide nanoparticles was found to be 48.16 µg mL⁻¹. Maximum concentration of

Fig. 15.11 DNA laddering of *Sesamum indicum* leaf DNA treated with different concentrations of ZnO nanoparticles. C control, ZnSO₄ zinc sulphate, M marker and ZnO—*L. aculeata*-mediated zinc oxide nanoparticles



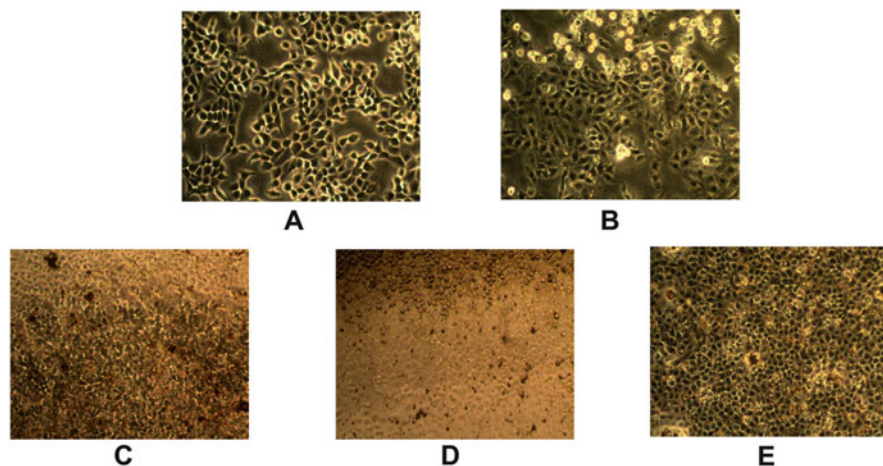


Fig. 15.12 Cytotoxicity effect of *Lantana aculeata*-mediated ZnO nanoparticles on SiHa cell lines. (a) $6.25 \mu\text{g mL}^{-1}$, (b) $12.5 \mu\text{g mL}^{-1}$, (c) $25 \mu\text{g mL}^{-1}$, (d) $50 \mu\text{g mL}^{-1}$ and (e) $100 \mu\text{g mL}^{-1}$

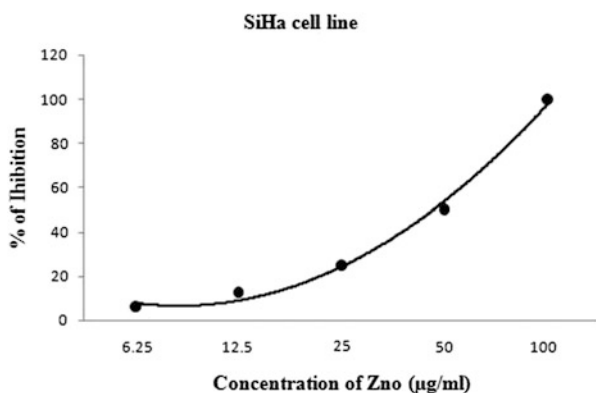


Fig. 15.13 Effect of *Lantana aculeata*-mediated ZnO nanoparticles on cell inhibition of SiHa cell line

zinc oxide nanoparticles ($100 \mu\text{g mL}^{-1}$) effectively inhibits the growth of cell by more than 98%. Sankar et al. (2013) reported the anticancer activity of *Origanum vulgare*-mediated silver nanoparticles and cytotoxic effects of green-synthesized *O. vulgare*-mediated silver nanoparticles against human lung cancer A549 cells.

15.5 Conclusion

The green synthesis method has prompted the improvement of biomimetic methodologies for the development of advanced nanomaterials. Biological synthesis of nanoparticle utilizing plants extract have been recommended as could reasonably be expected eco-friendly different option for chemical and physical methods. A simple, rapid biological procedure has been developed to synthesize ZnO nanoparticles using *Lantana aculeata* leaf extract. The synthesized nanoparticles were characterized by UV-Vis spectrophotometer, FTIR, XRD, EDX, FESEM, and HRTEM. The ZnO nanoparticles synthesized from biological method showed particles are spherical in shape with size range from 12 nm to 25 nm, respectively. *Lantana aculeata*-mediated zinc oxide nanoparticles were assessed in *Sesamum indicum* and SiHa cell line. ZnO used in this study were mainly nanosized but also showed a strong tendency to aggregate in spite of sonication of the suspension. DNA fragmentation as a marker for genotoxicity was determined by comet assay and DNA laddering. Results from this study demonstrate that ZnO nanoparticles were toxic to both plant and cancer cell lines.

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