ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.59085-59091, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF ANTI-GENOTOXICITY OF THE Tio_2 NANOPARTICLE BIOSYNTHESIZED FROM *TURBINARIA CONOIDES*

***Syed Ali, M., Anuradha, V., Sripriya, V., Yogananth, N. and Sheeba, H.**

PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, India

ARTICLE INFO

Article History:
Received 15th July, 2017
Received in revised form
08th August, 2017
Accepted 20th September, 2017
Published online 31st October, 2017

Key words:

Seaweed, Ames test, Anti-genotoxicity, Chromosome aberration.

ABSTRACT

The aim of the study was to investigate anti-genotoxic effect of TiO_2 nanoparticle biosynthesized from *Turbinaria conoides.* Genotoxicity studies were conducted for screening of Mutagenicity using Ames test, direct plate incorporation method using tester strain of *Salmonella typhimurium* viz., TA 98 and hence to prove this as an anti-genotoxicant. The Mutagenicity assay was performed with 3 dose levels (0.312, 0.625, 1.25, 2.5 and 5.0 mg/ml) using all two tester strains without metabolic activation system (S9 fraction). Inhibition in the background lawn or reduction in revertant colonies was observed at tested dose concentration in two tester strains without metabolic activation system. Furthermore works are to be carried out in future to find the exact mechanism of its geno-protective nature.

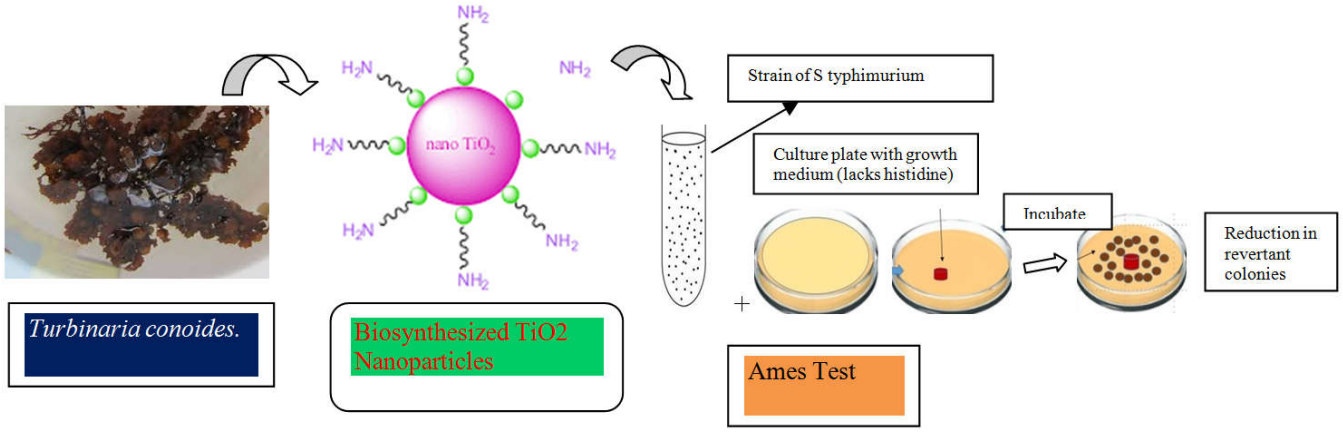
Citation: Syed Ali, M., Anuradha, V., Sripriya, V., Yogananth, N. and Sheeba, H. 2017. "Evaluation of anti-genotoxicity of THE Tio_2 nanoparticle biosynthesized from *Turbinaria conoides*", *International Journal of Current Research*, 9, (10), 59085-59091.

INTRODUCTION

In the recent years nanotechnology plays a vital role in the various fields like material science, biotechnology, analytical chemistry, engineering medicine etc. Nanoparticles are defined as a small object between 1-100 nm in size which behaves as a whole unit with respect to its transport and properties. Nanoparticle has multifunctional properties and very interesting applications in various fields such as medicine, nutrition and energy (Chandran *et al*., 2006). The biogenic synthesis of monodispersed nanoparticles with specific sizes and shapes have been a challenge in biomaterial science. Also, it has created remarkable advantages in the pharmacological industry to cure various bacterial and viral diseases (Song and Kim, 2009). Biosynthesis methods have more compensation over other classical synthesis procedures due to the availability of more biological entities and eco-friendly procedures. The rich biodiversity and easy availability of plant entities have been highly explored for the nanomaterials synthesis (Monda *et al*., 2011). Recently, the biosynthesis of nanosized particles, wires, flowers, tubes was reported successfully. These biological synthesized nanomaterials have potential applications in different areas such as treatment, diagnosis, development surgical nanodevices and commercial product manufacturing (Bar *et al*., 2009). Nanomedicine makes a huge impact in healthcare sector in treating various chronic diseases. Hence, eco-friendly synthesis of nanoparticles is considered as building blocks of the forthcoming generations to control various diseases (Cruz *et al*., 2010). Many previous reports are demonstrating that biosynthesized nanoparticle effectively controlled oxidative stress, genotoxicity and apoptosis related changes (Kim *et al*., 2007). Titanium dioxide Nanoparticles (TiO_2-NP) is currently used in many products. Depending on the type of particle, it may be found in sunscreens, cosmetics, drugs and paints. It is also being investigated for use of in removing contaminants from drinking water (Nanomaterials EPA is assessing in 2013). TiO_2 is that it is retained for a longer time within biological systems, non-toxic and stable without light irradiation. Genotoxicity is described as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens; they can cause mutations. Genotoxins include both radiation and chemical genotoxins (Leila Chekir-Ghedira *et al.,* 2013). There are three primary effects that genotoxins such as carcinogens, or cancer-causing agents, mutagens, or mutation-causing agents, or teratogens and birth defect-causing agents which affecting their genetic information. Anti-genotoxic compounds from various medicinal and aromatic plants are used for several years. Plant derived phenolic compounds has many anti-genotoxic properties which prevents the damage of DNA. The dietary intake of phytochemicals is effective against various dreadful diseases. Hence marine floral resource will offer a great scope for discovery of new drugs.

Corresponding author:* **Syed Ali, M.
PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, India.

Graphical Abstract



It is increasingly recognized that ocean contains a huge number of natural products and novel chemical entities with unique biological activities that may be useful in finding the potential drugs with greater efficacy and specificity for the treatment of human diseases (Haefnar, 2003). Plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles formation Aromal and Philip, (2012) (Monda *et al*., 2011). These primary and secondary metabolites are constantly involved in the redox reaction to synthesize eco-friendly nanosized particles. Based on the view of all the above, the present study is mainly focussed on synthesis, characterisation and evaluating the anti-genotoxicity of TiO2 nanoparticles of Turbinaria conoide. In the last few decades much study has been directed at popular medicine, with the aim of identifying natural products with therapeutic properties. By considering the significance of these bioactive compounds, our work aimed at conducting Genotoxicity studies for the screening of Mutagenicity using Ames test and to prove this as a precursor of drug and further investigations in future (Palaniselvam Kuppusamy *et al*., 2016). *Turbinaria conoides* is a brown algae belongs to the family of Sargassaceae, found mainly in tropical marine water and hence it has been used for fertilizer, children's fever, insect repellent, pesticides and has proved antibacterial, antioxidant and anticancer activity through synergistic effects. *Turbinaria conoides* is rich in bioactive compounds such as steroids, phenolics, flavonoids, reducing sugars, fucosterol, sulfated polysaccharides including fucoidan, neutral glucan, guluronic, alginic acid and it also possessed digestible proteins with mineral salts (K, Ca and Fe) and polyunsaturated fatty acids. The cytotoxicity of *Turbinaria conoides* was reported in ethyl acetate extract due to its oxygenated fucosterols (Arumugama Ponnan and Kavipriya Ramu, 2017). However, Anti-genotoxic potential of this species was yet to be explored. Hence, the nontoxic and highly stable charateristic of TiO_2 nanoparticle mooted us to investigate antigenotoxic effect of TiO_2 nanoparticle biosynthesized from *Turbinaria conoides*.

MATERIALS AND METHODS

The species of brown algae *Turbinaria conoides* were collected from Mandapam, South Coast of Tamilnadu, India. The algal materials were washed thoroughly with distilled water to remove debris and other associated biota. The samples were shade dried, powdered and stored at 4 C for further use.

Biosynthesis of TiO_2 nanoparticles from *Turbinaria conoides*

The *Turbinaria conoides* extract was prepared and boiled for 10 min with de-ionized water. The filtered broth extract was kept at 10 C for further experiments. For synthesis of TiO_2 NP's, 100 mL of $TiO(OH)_2$ (5mM) and 20 mL of the aqueous extract of *Turbinaria conoides* were stirred for 24 hours continuously. The formation of nanoparticles is confirmed by change in color to light green. The obtained precipitate was dried in a hot air oven at 120C for 1 hour. During drying, complete conversion of $TiO(OH)_2$ into TiO_2 took place.

Fig.1. ***Turbinaria conoides***

Characterization of Biosynthesised TiO_2 nanoparticles from *Turbinaria conoides*

The initial characterization of the biosynthesized TiO_2 NP was carried out after 24 hours between 300 nm to 800 nm using Perkin-Elmer lambda 25 UV-Vis spectrometer. The absorbance was taken after 5-fold diluting the sample with distilled water against distilled water as blank. Fourier transform infrared spectroscopy (FTIR) was used to identify the possible functional groups responsible for the reduction of the metal ions and capping of the bioreduced TiO_2 nanoparticles synthesized. The samples were analyzed on a ABB Horizon MB 3000 spectrum instrument in the diffuse reflectance mode operation with the scanning range of 4000-400cm-1 at a resolution of 0.7 cm^{-1} utilising the DTGS (Deuterium Triglyane sulphate) detector. In order to obtain good signal/noise ratio 512scans were recorded. The peaks obtained were plotted as % transmittance in Y axis and wave number (cm^{-1}) in x axis. Combined with the intuitive Horizon

MB™ FTIR software, the MB3000 will facilitate easy acquisition, processing and analysis of samples. Scanning Electron Microscopy is done for revealing the surface morphology of particles. Structural studies of TiO2 NPs were done by AURIGA- Cross beam FESEM (M/s Carl Zeiss, Germany). The characterization of the purified nanoparticles were conducted with an XRD 6000 X-ray diffractometry (shimadzu, Japan) operated at voltage of 40 kV and current of 30 mA with cu k radiation in θ 20 configurations. The crystallite domain size was calculated from the width of the XRD picks by assuming that they were free from non uniform strains and using the sherrer formula:

$$D = \frac{0.94}{\beta \cos \theta}$$

Where the D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X-ray wave length, β is the full width at of maximum (FWHM) and θ is the diffraction angle. To eliminate the additional instrumental broadening, the FWHM was curetted using FWHM from a large grained Si sample. β corrected = $(FWHM^2 \text{ sample} - FWHM^2 n)^{1/2}$ This modified formula is valid only when the crystallite size is smaller than 100 nm.

Ames test

Cells were treated with various concentrations of Ti-NPs as described in the toxicological study. Mutagenicity was assessed by the pre-incubation assay as described by Maron and Ames (Oesch and Landsiedel, 2012). Briefly, 100 µL of overnight cultures (1-2 × 108 cfu/mL) of strain TA98 were treated separately for 30 minutes at 37˚C (Dipanwita Dutta Deb *et al*., 2008) with various concentrations of Ti-NPs in the absence or presence of S9 mix. The Ti-NPs were suspended in sterile DW and were used at a final concentration of less than 5% (v/v). For the Ames test, the controls and Ti-NP-treated cells were mixed with 2 mL of sterile top agar (0.6% agar and 0.5% NaCl containing 0.5 mM histidine and 0.5 mM biotin) and poured onto minimal glucose agar plates (1 × Vogel-Bonner salts (0.2 g/L magnesium sulfate, 2 g/ L citric acid monohydrate, 10 g/L dipotassium hydrogen phosphate, and 3.5 g/L sodium ammonium phosphate), 2% glucose, and 1.5% agar). The plates were then incubated at 37˚C for 48 hours, after which revertants and surviving colonies were counted. Three independent experiments were conducted and each experiment consisted of three replicate plates for each treatment. The positive control used in the presence of S9 mix was 2-aminofluorene.

Plating Procedures

These procedures were used in the dose range-finding and mutagenicity assays. Each plate was labeled with the test item, test phase, tester strain, activation condition, and dose. Treatments in the absence of S9 were performed by adding 100 µl tester strain and 100 µl test or control to 2.5 ml molten diluted top agar (maintained at 45 ± 2 °C). The mixtures were vortexed and overlaid onto the surface of bottom agar dishes. After the overlay solidifies; the plates were inverted and incubated for 72 hrs at 37 ± 2 °C. After incubation the plates were evaluated for the condition of the background lawn for the evidence of cytotoxicity and test item precipitate in comparison with the control and the plates were evaluated for the number of revertant colonies.

Human lymphocytes culture and Chromosomal Aberration Studies

Heparinised blood samples (0.5 ml) was collected from healthy individuals and were placed in sterile culture flasks with 0.7 ml of RPMI1640 supplemented with fetal bovine serum (1.5 ml), antibiotic–antimycotic mixture (1.0 ml), phyto haemagglutinin (0.1 ml). The cultures are placed in incubator at 37 C for 24 hours. The whole blood was added to 10 ml of HiKaryoXL Medium in T-25 cm² flasks. The flasks were incubated at 37 C and 5% CO_2 for 67-72 hours. Peak mitotic index is most commonly observed at 67-72 hours. After 48 hours incubation, test compound (biosynthesis TiO_2)/positive mutagen of known concentration prepared in suitable vehicle or as such, will be added to the culture at a volume of 0.1 ml to achieve desired final concentration after all other constituents were added. Negative control cultures received vehicle-DMSO alone at a volume of 0.1 ml. Negative control culture without metabolic activation will receive only 0.5ml of phosphate buffer. The vials/centrifuge tube/flasks will be transferred to CO_2 incubator. The culture will be incubated at 37 ±1 °C and 5% CO_2 for 3-6 hours, flasks were transferred to labeled sterile 15 ml centrifuge tubes, centrifuged at room temperature at 1600 rpm for 5-10 min, supernatant will be aspirated gently and to the pellet a freshly made working growth medium RPMI 1640 without PHA-M were added. The total volume of the culture will be made up to 10 ml using the culture medium. The centrifuge tube/flasks were transferred to CO_2 incubator. The culture will be incubated at 37 ± 1 °C and 5% CO_2 for 18-21 hours. The 100µl of 10µg/ml of Colchicine and incubated for additional 2 hours. The entire content of the flask was transferred to a sterile centrifuge tube and centrifuged at 800-1000 rpm for 10 minutes. The supernatant was discarded and the pellet was suspended in 5ml of hypotonic 0.075M KCl solution and incubated in a water bath at 37°C for 15-20 minutes. The equal amount of freshly prepared ice cold fixative were added (Acetic acid: Methanol, 1:3 parts). The cells were centrifuged at 800-1000 rpm for 10 minutes. The fixative was removed by centrifugation and this process is repeated twice. The slides were prepared and they were stained with 3 % Giemsa stain solution in phosphate buffer (pH 6.8) for 15 min. At least 300 metaphases were scored in each slide for examining different types of abnormality according to standard protocol of Savage (Savage, 1979). Mitotic index (MI) was calculated by using formula, MI = number of dividing cells/total number of cells × 100, where MI, Mitotic index.

Statistical analysis

The Mean and standard deviation was calculated for each parameter. The data was analyzed by 'SPSS 17.0' software. Two way ANOVA was performed to determine significance of treatment. The mean separation was performed according to Duncan's New multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Plant, bioactive products and microorganisms provides the pharmaceutical industry with some of the most important sources of components for the research of new medications. In the last few decades much study has been directed at popular medicine, with the aim of identifying natural products with therapeutic properties. By considering the significance of these bioactive compounds, our work aimed at conducting

Genotoxicity studies for the screening of Mutagenicity using Ames test and to prove this as a precursor of drug and further investigations in future.

The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. It is based on the principle that molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. Characterization process of TiO_2 NPs includes UV-Vis absorbance measurement. TiO_2 NP dispersed in lake water gave a near-UV absorbance peak at 336nm. The dynamic aggregation process of TiO_2 NPs was monitored using a UV-Vis spectrophotometer at 378 nm, analyzing the sedimentation of the NPs in different water matrices.

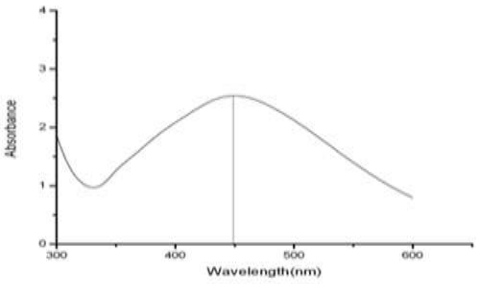


Fig. 1. The absorbance spectrum of TiO_2 nanoparticles showing maximum absorbance near 370nm

The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time. FTIR spectra of synthesized nanoparticles from *Turbinaria conoides* in the range of 400–4000 cm−1. The peaks at 3438.70 and 1640.26cm−1 in the spectra are due to the stretching and bending vibration of the -OH group. In the spectrum of pure TiO_2, the peaks at 523.88 cm1 show stretching vibration of Ti-O and peaks at 1416.38 cm-1shows stretching vibrations of Ti-O-Ti. Peaks at 3438.70 cm−1 indicates the presence of amines, Peaks at3287.11 cm−1 indicates the presence of Alkynes and Peaks at 3011.35 cm−1 indicates the presence of Aromatic rings.

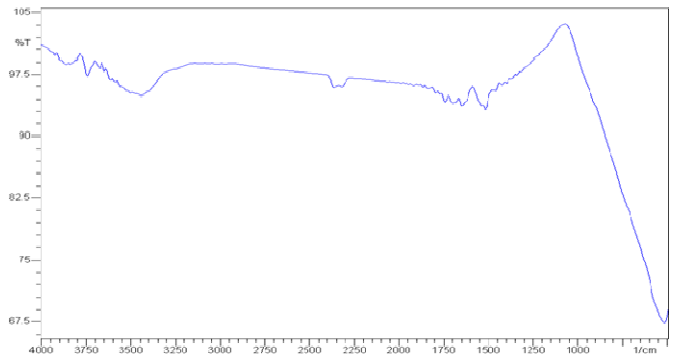


Figure 2. The FTIR absorbance spectrum of TiO_2 synthesis nanoparticles of *Turbinaria conoides*

The suspension of TiO_2 nanoparticles was dried inside a vacuum chamber for 24 hours so that a small amount of dry TiO_2 nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve confirmed that the nanoparticles are nothing but TiO_2. Interpretation of this XRD pattern reveals the existence of diffraction lines at low angles (5° to 75°). The TiO_2 nanoparticles showed the peaks of TiO_2 at 2θ = 38° and 44° that can be assigned to the (111) and (200) facets of TiO_2 respectively, which go very well with the values manipulated for face centered cubic structure of TiO2 nano-crystals (according to JCPDS: File No. 4-783). As the width of the peak increases size of particle size decreases, which resembles that present material in nano range.

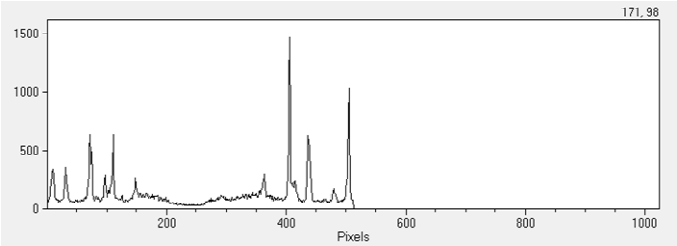


Figure 3. XRD Pattern of TiO_2 synthesis nanoparticles of *Turbinaria conoides*

Scanning Electron Microscopy is done for revealing the surface morphology of particles. Here, the bead for the SEM analysis was prepared by placing a drop of the silver nanoparticle suspension on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for a couple of hours. The particles on the top of the bead were scanned by Scanning Electron Microscope and the following image was obtained. The grain size, shape and surface properties like morphology were investigated by the Scanning Electronic Microscopy as shown in Figure 4. This image was observed within the magnification of 10 µm. The TiO_2 nanoparticles were showing irregular particle structure. The size was ranging from 60 nm to 80 - 100nm.

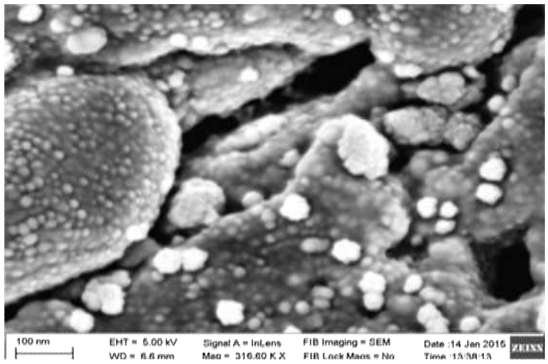


Figure 4. SEM image showing surface morphology of the TiO2 biosynthesis nanoparticles

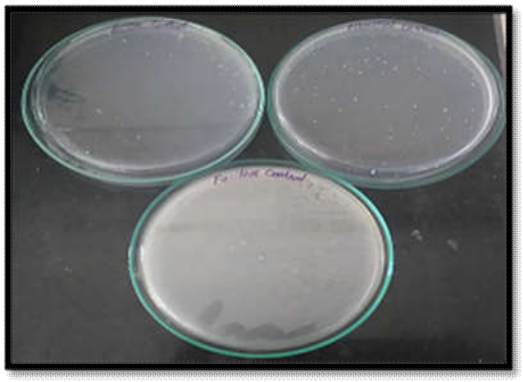


Figure 5. Normal, Positive control, DMSO for TA 98 (Set 1)

The mutagens (positive controls) treated without metabolic activation system showed a 3 fold increase of average revertant colonies per plate when compared with that of concurrent vehicle controls, thus exhibiting the ability to identify the mutagen by the tester strains (Fig 5). The mutagenicity assay was performed with three dose levels (0.312, 1.25, and 5.0 mg/ml) in the absence of metabolic activation system. Inhibition of background growth of non-revertant bacteria was not found at any of the three dose levels (Table 1).

Table 1. Mean Colony Count - Strain TA 98 spontaneous mutation

Test Item	Test Concentration	Histidine Revertant Colonies
	(µg/plate) / (mg/ml)	CFU/Plate
Positive Control	Sodium azide (1.5 µg/plate)	350
Synthesis TiO_2 nanoparticles	5	46
Turbinaria conoides (without S9)	1.25	74
	0.312	49

Table 2. Chromosome Aberration Assay of synthesis nanoparticles of *Turbinaria conoides* –without S9

Dose (mg/ml culture)	Total number of cells scored	Percent numerical aberration	Mean of structural aberration	Total number of aberration	Total no. of cells with aberration	No. of aberration per cell	Aberration frequency (%)
Negative control (DMSO)	100	0	0	0	0	0	0
0.312 mg/ml	100	0	0	0	0	0	0
	100	0	0	0	0	0	
1.25 mg/ml	100	0	0	17	5	3.40	5.5
	100	0	0	22	6	3.66	
5 mg/ml	100	0	0	40	16	2.51	18
	100	0	0	49	20	2.45	
Positive control	50	0	0	176	30	5.87	35
Mitomycin C	50	0	0	165	40	4.13	

NOTE- The results are average of three sets of experiments. a- P<0.05 compared to untreated, b- P<0.05 compared to Mytomycin

The study data represented in Table 3A showed no significant increase of His+ revertant colonies upon exposure to the synthesized nanoparticles of *Turbinaria conoides* at any of the dose levels incubated with any of the tester strains, without S9 addition, when compared to the respective controls in the mutagenicity assay. The average revertant colonies per plate treated with the control in the absence of metabolic activation system were found to be within the acceptance limits of the spontaneous revertant control values of respective *Salmonella* strains. The results showed no significant increase in the His+ revertant colonies following exposure to the samples at any tested concentration in any of the tester strains without S9 when compared with the negative control of each tester strain. Based on the above results, it is concluded that the synthesized nanoparticles of *Turbinaria conoides* were non-mutagenic at the dose levels ranging from 313 to 5000 µg/plate by the Ames bacterial reverse mutation assay in the absence of S9 mix, under the conditions of the test employed.

When human leukocytes were treated with TiO_2 nanoparticle of *Turbinaria conoides* alone at different doses; the incidence of cells having aberrants (including gap) in percentage of chromosomal aberration frequency maximum concentration of dose of 1.25 and 5 mg/ml no increase in number of CA were observed when compared to untreated. In contrast, the incidence of aberrant cells in each positive control group increased greatly as compared with each solvent group (P<0.001) Table 2. Present study, showed no significant increase of His+ revertant colonies following exposure to the *Turbinaria conoides* nanoparticle at any of the dose levels incubated with any of the tester strains, without S9 addition, when compared to the respective controls in the mutagenicity assay. It is well known that cyclophosphamide could cause chromosomal aberrations *in vitro* and *in vivo* (Kumpati Premkumar *et al.,* 2004, Lakshmi *et al.,* 2009) (Lakshmi and Auradha, 2006). It is mainly bio activated by liver cytochrome monooxygenase system into active metabolites (Sladek *et al.*, 1988; Peter Guengerich, 2004). These metabolites cross links with DNA and inhibit replication thus indirectly affecting cell growth (Thomas *et al.,* 1993; Oesch and Landsiedel, 2012). Our study, the *in vitro* chromosomal aberration test is to identify agents that cause structural chromosomal aberrations in cultured mammalian cells. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome type aberrations also occur. The *in vitro* chromosomal aberration test may employ cultures of established cell lines, cell strains or primary cell cultures. Chromosomal aberrations are the cause of many human genetic diseases and there is substantial evidence that chromosomal damage and related events causing alterations in oncogenes and tumor suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals.

Lakshmi *et al., in* 2009 studied the modulatory effects exerted by the extract of garlic (2mg/ml, 4mg/ml, 6mg/ml) against the mytomycin C induced genotoxicity in the human lymphocyte cultures in vitro by using three different doses of garlic extract and there was a significant decrease in the frequency of CA and SCE suggesting that the garlic extract modulates the Mytomycin C induced genotoxicity in a dose dependent manner. Similar to previous work in the present study with human peripheral leukocytes, three doses (0.312, 0.625, 1.25, 2.5 and 5.0 mg/ml) of TiO_2 nanoparticle was tested for antigenotoxicity against mytomycin C induced toxicity. The protective effect of *Ocimum sanctum* leaf extract (200µg/ml) against MMC induced DNA strand break, chromosomal aberrations and micronuclei formation *in vitro* were found to be 67% and 63% protection against DNA strand break, 68% and 59% protection against chromosomal aberrations and 63% and 68% protection against micronuclei formation (Dipanwita Dutta *et al.,* 2007) (Dipanwita Dutta Deb *et al*., 2008; Shakira Shaik *et al*., 2014). Similarly, chromosome aberration effect of synthesis of TiO2 particle of *Turbinaria conoides* maximum concentration (5mg/ml) maximum activity of CA aberration

26%. Previous research work focused on to synthesis of Titanium dioxide (TiO_2) nanoparticles from leave extract of Aloe Vera plant using Green synthesis method. The obtained TiO2 nanoparticles have been characterized by X-ray Diffractometer (XRD), Particle Size Analyzer (PSA), Scanning Electron Microscopy (SEM), Tunneling Electron Microscopy (TEM) and Thermo Gravimetric and Differential Thermal Analyzer (TG/DTA) for average crystallite size, average particle size, morphology, structure and thermal stability respectively (Ganapathi Rao, 2015) (Shakira Shaik *et al*., 2014). Based on the view of all the above, the present study is mainly focussed on synthesis, characterisation and evaluating the antigenotoxicity of TiO_2 nanoparticles of *Turbinaria conoides*. Previous studies revealed that synthesized *Ocimum* SNPs have potential anti-bacterial activity (Mallikarjuna *et al*., 2011; Singhal *et al*., 2011; Amit Kumar Mittal *et al*., 2013). On comparison with the stem, the leaf showed maximum synthesis because of their high phenolics, flavonoids (apigenin, luteolin) and tannins (Naheed *et al*., 2010). Further biosynthesis can be confirmed by appearance of brownish black colour upon addition of $AgNO_3$ which is due to surface plasmon vibration of synthesised nanoparticles (Jayaseelan *et al*., 2010; (Jayaseelan *et al*., 2012). The XRD pattern reveals sharp Bragg's peaks which are *Turbinaria conoides* seaweed thus promoting crystallisation of the synthesized TiO_2. The TiO_2 nanoparticles were successfully synthesized using green synthesis method. From XRD analysis average crystallite size of the sample was obtained 20 nm. It is observed that Tetragonal structure was formed. The average particle size was estimated 80-100 nm from particle size analyzer. The tetragonal irregular particle structure was observed in SEM image. The weight loss was measured by Thermo Gravimetric and Differential Thermal Analyzer (TG/DTA) curves as 0.98%. These above results showed that TiO2 particles were in the nano range. Thus it is known that administration of Mytomycin C causes increased cell damage which in turn leads to increased CA wherein co-administration of TiO_2 nanoparticles of *Turbinaria conoides* along with Mytomycin C reduced the frequency of Chromosome aberration and Ames test. Thus indicates the protective role of *Turbinaria conoides* seaweed. Thus it was inferred from the present study that the TiO_2 nanoparticles biosynthesized using *Turbinaria conoides* can be used as an efficient and potent drug owing of its anti-genotoxicty and anti-mutagenicity property.

Conclusion

Nanomedicine applications are used globally in healthcare setting for various treatments. The present study has demonstrated biosynthesised TiO2 nanoparticle from *Turbinaria conoides* possess potent Ames Test and anti-genotoxic activities, and the biological way of synthesizing nanoparticles has potential applications like cost effectiveness, eco-friendly and compatibility for large scale production. In conclusion, biosynthesised TiO2 from *Turbinaria conoides* were found to be non-mutagenic. However, further work has to be carried out in future to find other toxicological profile and to prove its safety use in various therapeutic area as a drug constituent.

REFERENCES

Ames BN, Lee FD, Durston WE. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc Natl Acad Sci USA*, 70(3):782-786.

Amit Kumar Mittal, Yusuf Chisti, Uttam Chand Banerjee, 2013. Synthesis of Metallic Nanoparticles Using Plant Extracts. *Biotechnology Advances*, 31:346-356.

Arumugama Ponnan, Kavipriya Ramu 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh) Kuetz. Clinical Phytoscience *International Journal of Phytomedicine and Phytotherapy*.

Dipanwita Dutta Deb, *et al*. 2008. In Vitro Safety Evaluation and Anticlastogenic Effect of BacoMind on Human Lymphocytes. *Biomedical and Environmental Sciences*, 21, 7-23 (2008).

Govindaraju, K., S. Tamilselvan, V. Kiruthiga and G. Singaravelu, 2010. Biogenic silver nanoparticles by Solanum torvum and their promising antimicrobial activity. *Journal of Biopesticides*, 3(I Special Issue) 394-399.

Hayashi, M. and Mac Gregor, J. 2000. In vivo rodent erythrocyte micronucleus assay. II some aspects of protocol design including repeat treatments, integration with toxicity testing and automated scoring. *Environ. Mol. Mutagen*., 35: 234–252. 16.

Hayashi, M., Mac Gregor, J. 2000. In vivo rodent erythrocyte micronucleus assay. II some aspects of protocol design including repeat treatments, integration with toxicity testing and automated scoring. *Environ. Mol. Mutagen*., 35: 234–252. 16

Jayaseelan, C., Rahuman, A.A., Kirthi, A.V., Marimuthu, S., Santhoshkumar, T., Bagavan, A., Gaurav, K., Karthik, L., Rao, K.V. 2012. Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. Spectrochim. *Acta A: Mol. Biomol. Spectrosc*., 90, 78–84.

Jiang W, Kim BY, Rutka JT, Chan WC. 2008. Nanoparticle-mediated cellular response is size-dependent. *Nat Nanotechnol*., 3(3):145- 150.

Lakshmi and Auradha 2006. Invitro studies on antiradical and antioxidant activities of fenugreek. (Trigonella foenum graecum) seeds. *Food Chemistry,* 103 (2007) 31, 37.

Manish Kumar, Paramjeet Kaur, Subodh Kumar, 2015. Antiproliferative and Apoptosis Inducing Effect of Non-Polar Fractions from Lawsonia inermis L. in Cervical (HeLa) Cancer Cells. Doi: 10.1007/s12298-015-0285-3.

Monda *et al*., 2011, S. Monda, N. Roy, R.A. Laskar, I. Sk, S. Basu, D. Mandal, N.A. 2011. Begum Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (Swietenia mahogani JACQ.) *leaves Colloid Surf. B*., 82:pp. 497-504.

Oesch F, Landsiedel R. 2012. Genotoxicity investigations on nanomaterials. *Arch Toxicol*., 86(7):985-994.

Palaniselvam Kuppusamy, Mashitah M. Yusoff, Gaanty Pragas Maniam, Natanamurugaraj Govindan, 2016. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological application-An undated report. *Saudi Pharmaceutical Journal*, Volume 24, Issued 4, July 2016, Pages 473-484.

Peter Guengerich, F. 2004. Cytochrome P450: What Have We Learned and What Are The Futures Issues? Drug Metabolism Reviews. Vol.36. No.2, pp. 159-197.

Savage, JRK. 1979. Classification and relationship of induced structural changes. *J Med Gen*., 13:103–122

Shakira Shaik, Lwankelekile Mkize, *et al.* 2014. Tetradenia Riparia Mediated Synthesis of Nano Gold Particles.

Wu X, Liu H, Liu J, haley KN, Treadway JA, Larson JP. 2003. Immunofluorscent labeling of cancer marker Her_2 and other cellular targets with semiconductor quantum dots. *Nat Biotechnol*., 21(1):41-6.

Yavuz, O., M. Cam, N. Bukan, A. Guven, and F. Silan, "Protective ¨ effect of melatonin on β-cell damage in streptozotocin-induced diabetes in rats," *Acta Histochemica*, Vol. 105, No. 3, pp. 261–266, 2003.

Zhang H. and Chen G. 2009. Potent antibacterial activities of Ag/Tio_2 nanocomposite powders synthesized by a one-potsol-gel method. *Environ Sci Technol*., 34(8):2905-10

****