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SYNTHESIZED IRON OXIDE NANO PARTICLES TREATED MCF-7 CELL LINES FROM AQUEOUS LEAVES EXTRACT OF *VITEX NEGUNDO*

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ABSTRACT

There are variety of nano particles systems currently being explored for cancer therapeutics, have attracted remarkable in targeted iron oxide nano particles for cancer therapy has increased dramatically in the past 10. However, synthesized iron oxide nano particles treated MCF-7 cell lines were assessed. DAPI staining method, MTT Assay were employed. The result showed that 50% cell viability loss at the dose of in 5.993. The treated cells showed distinct cellular morphological changes indicating unhealthy cells, whereas the control appeared normal in shape. Control cells were irregular confluent aggregates with rounded and polygonal cells.

KEYWORDS: Fe_2O_3 NPs, *Vitex negundo*, DAPI, MTT Assay, Control Cells, MCF-7, Cells Lines.

INTRODUCTION

Plants used in traditional medicine contain a vast array of substances that can be used to treatchronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health careneeds. The demand for more and more drugs from plant sources is continuosly increasing. It is therefore essential for systematic evaluation of plants used intraditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity. Literature survey of *V.negundo* revealed the presence of volatile oil, triterpenes, diterpenes, sesquiterpenes, lignan, flavonoids, flavones glycosides, iridoid glycosides and stilbene derivative. Though almost all parts *V.negundo* are used, the

extract from leaves and the roots is the most important in the field of phytomedicine and sold as drugs. The leaf extract is used in Ayurvedicand Unani system of medicine. Water extract of mature fresh leaves exhitibited anti-inflammatory, analgesic and antihistamine properties. Lignans, one class of natural compounds present in *V.negundo*, showed anti-cholinesterase activity in-vitro. However no studies were conducted to explore the effect of *V.negundo* extract against memory impairment in-vivo.

The evaluation of plant products on the basis of medicinal and therapeutic properties forms a platform for the discovery of newer drug molecules from different plant sources. From the innumerable plants being researched since time immemorial, *V.negundo* is important one. This plant of Verbenaceae family is commonly known as Nirgundi (Hindi) and five leaved chaste tree (English). *V.negundo* L.(Sambhalu(is an aromatic large shrub or small slender tree of about 3 meter in height with quadrangular branches. It is found in moist area, often on banks of rivers, throughout India up to an altitude fo 1500 meter, also grown in Mediterranean countries and Central Asia. Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant and hepatoprotective disorders. *V.negundo* commonly known as the five-leaved chaste tree. Herbal remedies are a type of alternative medicine that originates from plants and plant extracts. Used to heal illnesses and disease and to address psychological concerns, herbal remedies have been around for centuries and were the precursor to modern medicine. Herbal remedies are obtained from a wide variety of natural resources including plant leaves, bark, berries, flowers and roots.

MATERIALS AND METHODS

Collection and Identification

Fresh leaves *Vitex negundo* were collected from Permabur :, Chennai, Tamil Nadu, India, and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Science, Plant Anatomy Research Centre, West Tambaram, Chennai, India, as Lamiaceae with voucher specimen no: PARC/2017/235.

Taxonomical Classification

Kingdom : Plantae
Family : Lamiales
Genus : Vitex
Species : negundo

Bionomial name: ***Vitex negundo***

Common names: Pochotia (Assamese), Nirgundi, Nishinda, Samalu (Bengali), Lingeli (Bontok), Huang jing (Chinese), Five- leaved chaste tree, Horseshoe vitex, Chinese chaste tree (English), Lagundi (Filipino), Nagoda, Shamalic (Gujarati), Mewri, Nirgundi, Nisinda, Sambhalu, Sawbhalu (Hindi), Indrani (Malayalam), Nirgunda (Matrathi), Simali (Nepali), Banna (Punjabi), Nirgundi, Sephalika, Sindhuvara, Svetasurasa (Sanskrit), Nika (Sinhala), Lingad (Konkani), Chinduvaram, Nirnochchi, Nochchi, Notchi, Vellai- nochchi (Tamil), Vavili, Nalla-vavili (Telugu).

Fig. 1: ***Vitex negundo*** **leaves.**

Preparation of ***Vitex negundo*** **aqueous leaves extract**

About 100 g of fresh taxonomically authenticated healthy leaves of *Vitex negundo* were collected, washed thoroughly with running tap water and double distilled water, cut into fine pieces and shade dried for 10 days under dark condition. After drying the leaves were powdered using kitchen blender. The powdered leaves were soaked in the 200 ml of double distilled water for overnight in a fridge for 4°C and then the rinsed mixtures were boiled for 15 minutes. The extracts were cooled to room temperature and then filtered through Whatman filter paper (No.1).

Synthesis of Iron oxide nanoparticles

Fig. 1 shows the iron oxide nanoparticles synthesized by chemical precipitation method. The powder form of synthesized nanoparticles using the aqueous extracts of *Vitex negundo* black in colour (Fig. 1) and the intensity of color increased with time and dosage of plant extract it indicates the more growth of nanoparticles. The colour change is the most easy and

commonly used indication of the metal nanoparticles formation. The plant extract contained much organic content. Hence the mechanism study of iron oxide nanoparticles formation is a little difficult. However, the organic compound, which is present in the plant extracts act as a reducing as well as capping or binding agent to form iron oxide nanoparticles.

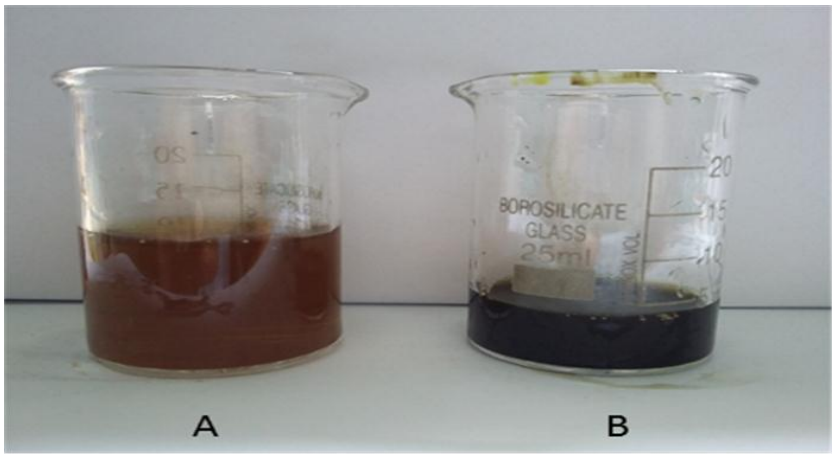


Fig. 1: A- *Vitex negundo* leaves aqueous extract

B- *Vitex negundo* leaves aqueous extract based synthesized iron oxide nanoparticles

Characterization of *Vitex negundo* iron oxide nanoparticles

Characterization of the synthesized iron oxide nanoparticles were carried out by UV-VIS absorption spectrum was recorded in the region 200-1000 nm using Varian Caty-5 UV-VIS-NIR Spectrometer. The FTIR spectra were recorded using Perkin Elmer Spectrum -1 in the range of 4000-450 Cm^{-1} at a resolution 1.0 Cm^{-1} based on KBr pellet technique, X-Ray Diffraction (XRD) patterns were recorded with a PAN Philips X'Pert ProX-ray diffractometer using CuKα radiation low angle diffract grams were recorded in the 2 theta range 5.0-80° with a 2 theta step size of 0.02° and a step time of 20 sec at each point, The surface morphologies were characterized by transmission electron microscopy (TEM) using Philips Techani 10 and high resolution scanning electron microscope (HRSEM) with energy dispersive X-ray analyser (EDAX) using FEI Quantum 200 MK II.

Preparation of Growth Medium

10 grams of Dulbecco's Modified Eagle's Medium (DMEM) was dissolved in 990 ml of sterilized double distilled water. To this solution, 1.5 g of sodium bicarbonate and 10 ml of streptomycin were added and mixed thoroughly. Later this medium was filtered using membrane filter (0.22 µm), dispensed into sterilized container and stored at 4°C. Fetal Bovine Serum (FBS) (10%) was added to this medium and used for cell culture.

Cell Line and Passage

The cells were grown in T-75 culture flask containing DMEM supplemented with 10% FBS and the flask was placed at 37°C in humidified incubator with 5% CO_2. When the cells reached 70-80% confluent, the spent medium was discarded and the monolayer was rinsed with Phosphate Buffered Saline (PBS). Trypsin-EDTA solution was added and placed in incubator for 2 min. After incubation, 5.0 mL of growth medium was added to the flask and mixed gently. Then it was transferred into a 15 ml falcon tube and centrifuged at 1000 rpm for 5 min. The supernatant was carefully aspirated and the pellet was gently resuspended in 2.0 ml of growth medium. The cells were diluted with appropriate volume of growth medium and the aliquot was transferred to a new culture flask at the density of $2\times10^3/cm^2$ and kept back to controlled environment for large scale production.

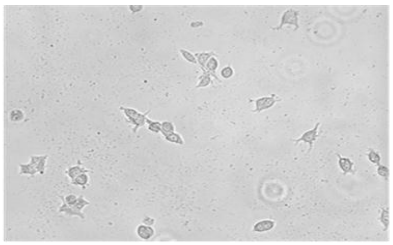
RESULTS AND DISCUSSION

Cell Morphological Study

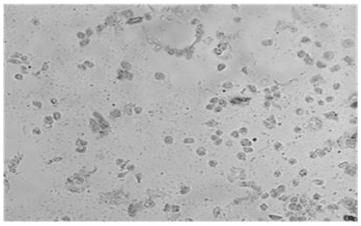
The morphological changes of aqueous extract based synthesized iron oxide nanoparticles treated MCF-7 cell lines were assessed by using light microscopy. Cancer cells (1×10^6 cells/ml) were plated in 100 mm dishes and incubated for 24 h under controlled environment. Then, the spent medium was removed, followed by addition of fresh medium with or without crude extract at an inhibitory concentration and incubated for 24 and 48 h. After incubation, the cells were visualized under Radical inverted light microscope at 20X magnification.

Nuclear Staining and Apoptotic Morphology (DAPI Staining Method)

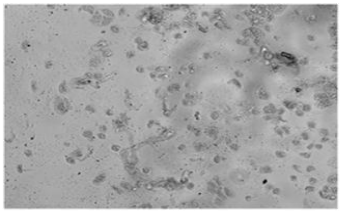
The morphological investigations on aqueous extract and aqueous extract based synthesized iron oxide nanoparticles with MCF-7 cells are depicted in Fig.2. It shows broken up cells leading to apoptotic bodies. Morphological evolution of the MCF-7 cell lines treated with aqueous extract and aqueous extract based synthesized iron oxide nanoparticles at IC_{50} concentration of 6.009 µg/ml for 24 hrs (aqueous extract) and iron oxide nanoparticles (5.993 µg/ml) at 48 hrs using phase contrast microscopy revealed morphological features of apoptotic cells, membrane blebbing and shrinkage of the cytoplasm and DNA fragmentation of the nuclear chromatin in comparison with control. ethanolic extract of *Vitex negundo* induced MCF-7 cells apoptosis was confirmed by using DAPI, which easily penetrate to the apoptotic cells. The apoptotic cells loose the membrane phospholipid, which leaves phosphatidylserine on the outer surface the plasma membrane.



Control MCF-7 cells



Aqueous extract ic50



Iron oxide nanoparticles ic50

Anticancer study of *Vitex negundo* leaves extract and aqueous extract synthesized iron oxide nanoparticles

The cell viability was assessed using anti-proliferation activity by MTT assay for 24 hrs and 48 hrs. The anti-proliferation increased with increase in concentration. The anti-proliferation activity was noted in the *Vitex negundo* leaves extract and aqueous extract synthesized iron oxide nanoparticles which is directly proportional to the concentration. The MTT results showed that 50% cell viability loss at the dose of in 5.993 48 hrs of incubation in iron oxide nanoparticles (Table 4) and the treated cells showed distinct cellular morphological changes indicating unhealthy cells, whereas the control appeared normal in shape (Fig. 1). Control cells were irregular confluent aggregates with rounded and polygonal cells.

Table 1: Per cent cell viability of MCF-7 cells for 24 and 48 h when treated with *Vitex negundo* leaves extract and aqueous extract synthesized iron oxide nanoparticles.

Concentration µg/ml	Aqu. extract 24 hrs	Aqu. extract 48 hrs	FeNPs 24 hrs	FeNPs 48 hrs
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0
2	94.382±0.287 (-5.618)	86.438±1.319 (-13.562)	84.47±0.402 (-15.53)	78.744±0.612 (-21.256)
4	89.048±0.633 (-10.952)	78.666±0.719 (-21.336)	72.578±0.295415 (-27.422)	58.674±0.671 (-41.33)
6	78.996±0.389 (-21.004)	68.438±0.298 (-31.562)	59.638±0.878 (-40.362)	36.582±0.527703 (-63.418)
8	71.098±0.512 (-28.902)	60.094±0.796 (-39.906)	45.582±0.303 (-54.418)	8.806±0.918 (-91.194)
10	61.392±0.5777 (-38.608)	42.742±0.414 (-57.258)	31.282±0.490 (-68.718)	0±0 (-100)
IC_{50}	6.009	7.971	6.057	**5.993**

Values are mean ± S.E. of six individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control

CONCLUSION

Medicinal plants, which are the backbone of traditional medicine, have in the last few decade been the subject for very intense pharmacological studies, the value of medicinal plants as potential sources of new compounds of therapeutics value and as a sources on new compounds of therapeutics value and as sources of lead compounds in the drug development. There arises a need therefore to screen medicinal plants for bioactive compounds as a basus for further pharmacological studies. According to the thorough study of the available literature it is quite obvious that the importance of *V.negundo* in traditional system of medicine is of utmost significance. Almost all parts of the plant are use in preparing herbal medicines. The plant is known to possess anticancer, antimicrobial, antifeedant, anti-inflammatory, antihyperpigmentation, hepatopropective, antihistaminic, analgesic and related activities. Scientifically explored exhaustive reports of the plant, their medicinal properties and active chemical constituents have a role in the management of various human ailments.

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