A

PROJECT REPORT

ON

THE NEXT GENERATION OF CANCER TREATMENT THROUGH MODERN THERAPY USING NATURAL DRUG EMBEDDED WITH NIOSOME RECEPTORS

SUBMITTED BY

P.M. SACHIN SUBASHINI(U19BR042)

P. DIVYA DARSHINI(U19BR037)

BIOTECHNOLOGY SPECIALIZATION IN GENETIC ENGINEERING

BHARATH INSTITUTE OF HIGHER EDUCATION AND RESEARCH

(Declared under section 3 of the UGC Act, 1956)

SELAIYUR, CHENNAI-600073, TAMIL NADU, INDIA[i]

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Submitted by

P.M. SACHIN SUBASHINI(U19BR042)

P. DIVYA DARSHINI(U19BR037)

In partial fulfillment for the requirement of the degree

Of

Bachelor of Technology

in

Biotechnology specialization in Genetic Engineering

Under the guidance of

V.INDIA

BHARATH INSTITUTE OF HIGHER EDUCATION AND RESEARCH

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DEPARTMENT OF GENETIC ENGINEERING

This is to certify that "**THE NEXT GENERATION OF CANCER TREATMENT THROUGH MODERN THERAPY USING NATURAL DRUG EMBEDDED WITH NIOSOMES RECEPTORS** is a bonafide work done by **P.M. SACHIN SUBASHINI(U19BR042), P. DIVYA DARSHINI(U19BR037)** for the major project in Genetic Engineering during the academic year 2022-2023.

Internal project guide
(V. INDIA)

H.O.D
(Dr .L. JEYANTHI REBECCA)

Submitted for the viva voce held on 10/5/23 at BIHER

Internal examiner

External examiner

AviGen BioTech Pvt Ltd.

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CERTIFICATE

This is to certify that this dissertation entitled **"THE NEXT GENERATION OF CANCER TREATMENT THROUGH MODERN THERAPY USING NATURAL DRUG EMBEDDED WITH NIOSOME RECEPTORS"** submitted to Department of Biotechnology Specialization in Genetic Engineering, Bharath Institute of Higher Education and Research, Chennai, in partial fulfillment for the award of the degree of B.Tech. Genetic Engineering is a record of original work done by **P.M. SACHIN SUBASHINI (Reg. No. U19BR042)** and **P. DIVYA DARSHINI (Reg. No. U19BR037)** at AviGen BioTech Pvt Ltd., under my supervision and guidance from March 2023 to April 2023. This project has not been submitted to any other degree.

Date : 09.05.2023
Place : Chennai

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DECLARATION

I hereby declare that the project report entitled "**THE NEXT GENERATION OF CANCER TREATMENT THROUGH MODERN THERAPY USING NATURAL DRUG EMBEDDED WITH NIOSOMES RECEPTORS**" submitted to BIHER, Chennai in partial fulfillment UG for the award of degree of Bachelor of Technology in Biotechnology specialization in Genetic Engineering is the record of the original work carried out by me under the guidance of **V. INDIA**

I further declare that the result of the work has not been submitted to any other university or institute for the award of any degree or diploma.

P. Divya

Signature of student

Place: Chennai

Date:

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ABSTRACT

Cancer is the body's uncontrolled proliferation of aberrant cells. Cancer is now regarded as a human tragedy and one of the most common diseases worldwide, and the mortality rate associated with cancer is rising. These innovative methods to prevent and treat such a fatal illness seems to be important. Targeted medicines, such as small molecule inhibitors, have fundamentally altered the way that cancer is treated over the past 10 years. These medications are currently a part of the treatment for many common cancers, such as lymphoma, leukemia, multiple myeloma, breast, colorectal, lung, and ovarian cancers. Targeted treatments have different modes of action and toxicity than conventional cytotoxic. The study of phytochemical analysis to check the antioxidant activity for the synthesis of natural drug and to target the anticancer study by Invitro model. Control survival and death of cancerous cell are important strategies in the management and therapy of cancer. Small molecule inhibitors are prone to several medication interactions. With the advent of targeted therapy, there are now additional concerns about how to best treat each patient's tumor. The evaluation of drug efficacy and toxicity as well as the financial aspects of cancer treatment.

KEYWORDS:

Niosomes, cytotoxic, antioxidant, natural drug, anticancer, invitro, tumor.

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SL.NO	ABBREVATIONS	FULL FORM
1	DPPH	α-diphenyl-β-picrylhydrazyl (DPPH)
2	DMEM	Dulbecco's Modified Eagle Medium)

1.INTRODUCTION

Terminalia chebula, also known as **Haritaki** in Hindi, **Kadukkai** in Tamil, and **Inknut** in English, is a medical marvel with a variety of medicinal purposes. It has several, diverse medical properties, earning it the title of "king of medicine." It has been widely employed in Ayurveda, Unani, and homoeopathy.

The number of disorders that Haritaki may cure is astounding; it is used to treat arthritis, dental problems such as cavities and bleeding gums, skin problems such as acne and dermatitis, and wound healing! Kadukkai is also used to treat neurological disorders such as Alzheimer's, to treat constipation, to decrease hemorrhoids when applied externally, and to efficiently heal ulcers.

Kadukkai is also used to treat and prevent kidney stones, to improve digestion, to treat coughs, sore throats, and headaches, and to treat eye inflammations when used as an eye rinse. Finally, it is widely used to boost immunity, cure fevers, and lower cholesterol and blood sugar levels.

The botanical name is Terminalia chebula. It is a member of the kingdom Plantae, the order Myrtales, the family Combretaceae, the genus Chebula, and the species Chebula.

The number of tannins in Haritaki fruit, which range from almost 32% to 34% and are of the pyrogallols type, can vary depending on the region in which it is grown. Gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, and casuarinin are only a few of the over 14 tannins that have been discovered in the fruit. Haritaki also contains poly phenols like corilagin, galloyl glucose, punicalagin, terflavin A, and maslinic acid.

The three main fatty acids present are palmitic acid, linoleic acid, and oleic acid. Triterpenoid Glycosides: Some of the glycosides present in Haritaki are chelosides I and II, arjunin, and arjun glucoside. Rutin, quercetin, luteolin, and isoquercetin are a few of the flavonoids that can be discovered.

Haritaki also possesses anticancer properties. Haritaki extract was found to be effective against human breast cancer cells, human osteosarcoma cells, human prostate cancer cells, and non-tumorigenic immortalized human prostate cells in a study.

Haritaki extract reduced the number of cancer cells, slowed cell proliferation, and caused cell death. The gallic acid present in Haritaki also inhibits the growth of esophageal cancer cells, as demonstrated by this study.

Various chemicals found in medicinal plants, including ascorbic acid and polyphenolic compounds, are to blame. By chelating with various metals, they must limit lipid peroxidation by inactivatin Any agent that prevents or reduces oxidative damage to a target molecule is an antioxidant, in the broadest sense. Because of their redox hydrogen donators, antioxidants have the ability to scavenge free radicals.

The natural (plants) and manufactured (butylated hydroxyl toluene, butylated hydroxyl anisole, and tetra butyl hydro quinone) antioxidants can scavenge free radicals. However, the natural antioxidants are now used instead because they are thought to be safer and come with no negative side effects. lipoxygenase and the relevant free radical.

The human body has a variety of active free radicals that primarily govern metabolic activity and play a significant role in a number of disorders, including heart disease, cancer, neurological diseases, and the ageing process.

The presence of common and natural substances including carbohydrates, phenols, saponins, terpenoids, alkaloids, flavonoids, and other elements is demonstrated by the phytochemical constituents and activity found in the plant portion.

The DPPH free radical scavenging method is the initial method for determining whether a substance, an extract, or other biological sources have antioxidant potential. The prospective compound or extract is combined with DPPH solution using this straightforward approach, and then the absorbance is measured after a predetermined amount of time. Although the fundamental strategy is the same across all of them, the method has undergone various modifications to suit the requirements due to the development and sophistication of instrumental techniques.

Because free radicals are inevitably produced in biological systems, it's crucial to understand the antioxidant content and effectiveness of these systems. Blois (1958) created the DPPH assay method with the intention of determining the antioxidant activity similarly by employing a stable free radical.

DPPH, or 1,2-diphenyl-picrylhydrazyl, the assay is based on the evaluation of the antioxidants' ability to scavenge it. By obtaining a hydrogen atom from antioxidants and converting it to the equivalent hydrazine, the odd electron of the nitrogen atom in DPPH is lowered (Contreras-Guzman and Srong, 1982).

Unlike most other free radicals that dimerize, DPPH is categorised as a stable free radical due to the spare electron's delocalization across the whole molecule. When DPPH solution is combined with something that may donate a hydrogen atom, the reduced form is formed with a loss of violet colour. The rich violet tint is also due to delocalization.

A technique developed to extract the most antioxidant power from the sample is used to measure antioxidant activity. The target substrate, the source of ROS, and the antioxidant activity are all strongly absorbed by the stable radical. An antioxidant may accelerate damage to other biological molecules while protecting lipids from oxidative degradation.

As an alternative to liposomes, niosomes are vesicles made of non-ionic surfactants that are biodegradable, more harmless, more stable, and less expensive. Drug molecules having a wide variety of solubilities can be accommodated by the structure's hydrophilic, amphiphilic, and lipophilic moieties. These might serve as a depot, gradually releasing the medication. By delaying clearance from the circulation, shielding the drug from its biological environment, and limiting its effects to target cells, drug molecules can also operate therapeutically better.

While liposomes are made from double-chain phospholipids (neutral or charged), niosomes are made from uncharged single-chain surfactant and cholesterol. In comparison to niosomes, liposomes have a significantly higher concentration of cholesterol. As a result, liposomes are less effective at encasing drugs than niosomes. As a result of their propensity for oxidative breakdown, phospholipids, one of the constituents of liposomes, are chemically unstable and require careful treatment and storage. Additionally, the quality of naturally occurring phospholipids varies.

The thin-film hydration technique, commonly known as the hand-shaking method, involves dissolving cholesterol and surfactant in a volatile organic solvent before transferring the mixture to a rotary evaporator. A thin layer of the solid mixture is then applied to the flask wall following evaporation. The target drug-containing aqueous phase is then used to hydrate the dried layer. This procedure can be done in room temperature with gentle agitation.

The "bubble" method allows for the production of niosomes without the use of organic solvents. At 60°C, a buffer containing surfactant and cholesterol is homogenized and "bubbled".

One of the first in vitro bioassay techniques used to gauge a substance's toxicity to diverse tissues was the cytotoxicity assay. Testing for in vitro cytotoxicity is an essential tool for screening and evaluating compounds' safety as well as ranking them.

Using the MTT assay, 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide was used to evaluate the cytotoxicity and anticancer activities in vitro []. The same methodology was used in two separate studies to evaluate the cytotoxicity and anticancer effectiveness of the test medicine as well as the percentage of cell viability and mortality.

Mitochondrial activity is determined by how well living cells convert MTT into formazan crystals. This assay is frequently used to detect the in vitro cytotoxic effects of medicines on cell lines or primary patient cells since for the majority of cell types the total mitochondrial activity is proportional to the number of viable cells. The procedure of the assay is discussed in this chapter, along with significant factors pertinent to each assay step, its limits, and potential applications.

The Kadukkai extract significantly inhibited adhesion and cell proliferation in cancer cell lines in vitro and caused the loss of membrane integrity as measured by the trypan blue exclusion test.

Two extremes of a spectrum of cell death are represented by apoptosis and necrosis. This spectrum has a wide range of variants. According to Leist and Jäättelä (2001), "apoptosis-like programmed cell death" refers to a type of cell death that exhibits some of the characteristics of apoptosis, such as chromatin condensation and the presence of PS on the outer leaflet of the

cell membrane. "Necrosis-like programmed cell death" refers to programmed cell death with different degrees of other apoptotic characteristics but without chromatin condensation. In some instances of this kind of planned cell death, caspase-1 and caspase-8 have been linked (Leist and Jäättelä, 2001). The term "paraptosis" refers to a type of cell death that requires gene expression but differs morphologically from both apoptosis and necrosis (Sperandio et al.2000)

Additionally, in vitro-cultured apoptotic cells eventually experience "secondary necrosis". Apoptotic cells eventually shut down their metabolism after prolonged incubation, lose the integrity of their membranes, and leak the contents of their cytoplasm into the culture medium (Riss and Moravec, 2004). Therefore, some of the morphological phenotypes connected to necrosis may be present in cells that have started the apoptosis process. In order to identify the process of cell death in their experimental system, researchers must look at a number of biochemical markers at precisely chosen time points because programmed cell death can take many different morphological and biochemical forms.

Necrosis, which is first characterised by a loss of cell membrane integrity, are the events of apoptosis. The necrotic cell's cytoplasm and mitochondria enlarge, and eventually the cell and many of its internal organelles lyse. There is no production of vesicles or apoptotic bodies, and necrosis frequently affects clusters of nearby cells. Inflammatory macrophages phagocytose the remains of necrotic cells.

The initial sign of apoptosis is a change in the cell's refractive index, which is followed by cytoplasmic shrinkage and nuclear condensation (Hen Gartner, 1997). Depending on the kind of cell, the cell membrane starts to develop blebs or spikes (protrusions of the cell membrane). Eventually, these blebs and spikes split from the dying cell to create "apoptotic bodies". Additionally, phospholipid asymmetry in the cell membrane is lost in apoptotic cells, and phosphatidylserine (PS) is seen on the outer leaflet (Williamson, 2000).

Most DNA-related functions, such as transcription and replication, halt due to chromatin condensation and only resume following chromatin decondensation in the early G1 phase. However, it has been demonstrated that RNA polymerase I component association with rDNA loci begins during mitosis. Because of its spatial organisation into tightly packed higher-order structures, chromatin condenses to a smaller volume. Specific histone alterations, such as the phosphorylation of histone H1 and H3, take place during mitosis and aid in the chromosomes' individualization and condensation.

2.OBJECTIVE

Collection of samples in powder substance (commonly known as Kadukkai). Extraction of different sample in Water and Ethanol. To study the Phytochemical assays by DPPH and to check the antioxidant activity. Synthesis and Characterization of niosomes by UV method. Natural Drug incorporated with niosomes. To study the antioxidant activity of niosomes. To study the cytotoxicity of natural drug/niosomes using Invitro. To target the Anticancer study by Invitro model. Apoptosis analysis of natural drug. To study the chromatin condensation by DAPI stain method.

3.REVIEW OF LITERATURE

3.1 ANWESA BAG.et.al (2013)

Since the beginning of civilization, medicinal plants have been an integral element of human society in the fight against disease. Because of its exceptional therapeutic abilities, Terminalia chebula Retz. (Family Combretaceae), known in Tibet as the "King of Medicine," is constantly at the top of the list of "Ayurvedic Materia Medica." The entire plant is highly therapeutic and has historically been used to heal a variety of human illnesses. According to certain mythology, this plant was used to treat asthma, sore throats, bleeding piles, ulcers, gout, heart and bladder conditions, as well as vomiting, hiccoughing, diarrhea, and dysentery. The plant has been shown to have numerous pharmacological and medicinal properties, including antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, radioprotective, cardioprotective, anti-arthritic, and gastrointestinal motility and apoptotic activity. The therapeutic efficacy of

Terminalia chebula, a well-known herbal treatment in South-East Asia and India, has so far been documented. The current information in this study focuses in particular on the phytochemistry, various pharmacological, and therapeutic capabilities of Terminalia chebula Retz. and several of its isolated components, as well as an assessment of their safety. This could serve as motivation for a thorough assessment of the plant's efficacy as a treatment for human diseases as well as fill in any gaps in the literature currently in use. This could also open up a wealth of opportunities for researchers working to verify traditional medical claims and create safe and effective botanical medicines.

3.2 Sahira Banu, Lji Joarics (2015)

The pharmaceutical industry values plants for their extensive structural variety and diverse spectrum of pharmacological effects. Phytochemicals are the substances found in plants that are biologically active. These phytochemicals come from many different plant parts. Direct medicinal agents are derived from these phytochemicals. They act as a starting point for the development of more sophisticated semi-synthetic chemical substances. This study primarily discusses plant collecting, active component extraction from diverse plant parts, and qualitative and quantitative analysis of phytochemicals. The substances that are naturally present in plants are known as phytochemicals. These phytochemicals are becoming more and more well-known today thanks to their numerous medical applications. Asthma, arthritis, cancer, and other disorders are all greatly aided by phytochemicals. contrary to pharmaceutical chemicals There are no negative side effects from these phytochemicals. They can also be referred to as "manfriendly medicines" because phytochemicals treat illnesses without harming humans.

3.3 Mohammed Fazil Ahmed et.al (2012)

The purpose of this study was to analyse the phytochemical composition and antioxidant activity of kadukkai leaf extracts. The kadukkai leaves were

extracted, and the phytochemical screening revealed the presence of certain active components including alkaloids, tannins, saponins, phenols, and more. steroids, terpenoids, flavonoids, and glycosides. The total phenolic content and antioxidant activity of the ethanolic leaf extract were also assessed using a DPPH radical scavenging test. According to the findings of the current study, Melia azedarach's ethanolic leaf extract, which contains the largest concentration of phenolic compounds, exhibited greater anti-oxidant activity than petroleum ether and aqueous extracts. The presence of hydroxyl groups in phenolic compounds may be the cause of the strong scavenging ability.

3.4 OM P. SHARMA et.al (2008)

A typical antioxidant assay is based on the scavenging of the DPPH free radical. For this assay, a number of methods have been used, leading to variations in the outcomes from different laboratories. We discuss the inconsistent results of the methods used by various researchers and suggest a uniform method that falls within the spectrophotometric sensitivity range. three widely used antioxidants. The reaction medium has a significant impact on the free radical scavenging.

3.5 Pei Ling yeo et.al (2017)

The localization of pharmacological molecules to sick locations is made possible by target-specific drug-delivery systems. Carriers include immunoglobulins, serum proteins, synthetic polymers, liposomes, and microspheres are used in a variety of medication delivery systems. The bilayer of the niosomes' vesicular system A nonionic surfactant-based structure can increase a drug's bioavailability for a time period to a specific area. Niosomes are more effective at encapsulating lipophilic or hydrophilic pharmaceuticals due to their amphiphilic composition. Cholesterol and other substances can be utilised to keep the niosomes' structure firm. The essential characteristics of niosomes, including their structural elements, preparation techniques, restrictions, and contemporary applications to a number of disorders.

3.6 JAYACHANDRA KUNCHA et.al (2019)

Natural substances have been used as anticancer agents for a very long time, first in folk medicine and then in allopathic and conventional medicine. Many

medications in use today are derived from healing herbs. The majority of the world's population has recently observed a transition away from synthetic to natural medicine, with medicine returning to its natural state. Herbal medicines have been utilised to treat a variety of disorders since ancient times. Ayurveda, Siddha, Unani, Homoeopathy, Yoga, and Naturopathy are some of the Indian traditional medical systems that are still used today for health maintenance by people all over the world. More research is being done on phytomedicine these days, and a number of botanical preparations are being assessed for their potential therapeutic efficacy.

3.7 AV BADARINATH et .al (2010)

Several current in-vitro techniques for detecting antioxidant characteristics are discussed together with in-vitro approaches to assess the antioxidant defense system. the various techniques utilised to carry out in-vitro antioxidant activity. It places emphasis on the method's simplicity, the amount of time needed, and the instrumentation needed, which forces us to choose the best way to use to measure antioxidant property based on the opportunities available. It gives a quick overview of the benefits of several methods and which is the most popular way currently being utilised for efficient analysis.

3.8 PEER J. (2020)

Anticancer compounds that may be less likely to cause adverse effects or weaken the resistance of current antitumor medications, various in vitro assays and various human cancer cell lines, including ovarian cancer, were used to evaluate the activities. The kadukkai extract significantly inhibited adhesion and cell growth in cancer cell lines in vitro, as determined by the evaluation of the clonogenic assay, and produced a loss of membrane integrity as measured by the trypan blue exclusion test.

3.9 LEIST and JAATTELA (2001)

Two extremes of a spectrum of cell death are represented by apoptosis and necrosis. This spectrum has a wide range of variants. According to , "apoptosis-like programmed cell death" refers to a type of cell death that exhibits some of the characteristics of apoptosis, such as chromatin condensation and the presence of PS on the outer leaflet of the cell membrane. "Necrosis-like programmed cell death" refers to

programmed cell death with different degrees of other apoptotic characteristics but without chromatin condensation. In some instances of this kind of planned cell death, caspase-1 and caspase-8 have been linked (Leist and Jäättelä, 2001). The term "paraptosis" refers to a type of cell death that requires gene expression but differs morphologically from both apoptosis and necrosis (Sperandio et al.

3.10 SAGAR B. KEDARE (2011)

α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. This is the simplest method, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period. However, with the advancement and sophistication in instrumental techniques, the method has undergone various modifications to suit the requirements, even though the basic approach remains same in all of them. This article presents a critical review on various developments to the DPPH method.

3.11 GEORGE FOTAKIS et.al (2006)

Invitro cytotoxicity assays are exposed to metals cytotoxicity, a protein assay, the neutral red assay and the MTT assay the MTT and protein assay were employed were as the neutral assay revealed early cytotoxicity starting after incubation. MTT assay reveals cytotoxicity due to exposure after 3h were as no such effect is seen. MTT assay at lower concentration are required for detection of toxicity and being most sensitive in detection cytotoxic events. Invitro cytotoxicity use end point parameter such as cell viability, cellular proliferation, cell membrane damage. DNA synthesis as the indicators. It often measures the extend of cell death which alter certain function of cells but do not result in cell death invitro cell viability and cytotoxic assay with cultured cells are used as chemicals for drug screening. This assay is used in both compound toxicity and tumors cell growth inhibition during drug development.

3.12 E SAFARZADEH (2014)

Cancer is the body's unchecked proliferation of aberrant cells. Cancer is now regarded as a human tragedy and one of the most common diseases worldwide, and the mortality rate associated with cancer is rising. Finding innovative methods to prevent and treat such a fatal illness seems to be important. Controlling malignant cell survival and death are crucial methods in the treatment and management of cancer. Through the induction of apoptosis, anticancer agents should kill the cancerous cell with the fewest negative effects on normal cells. Both in healthy tissues and damaged tissues, apoptosis is referred to as programmed cell death. This process involves morphological changes in cells, including rapid cell condensation and budding as well as the development of membrane-enclosed. Organelles from apoptotic corpses were well-preserved. One of the most crucial indicators of cytotoxic anticancer drugs is the induction of apoptosis. Some organic substances, such as those found in plants, cause apoptotic pathways to be stopped in cancer cells through a number of processes. According to numerous polls, persons with cancer frequently use herbal remedies or herbal products. As plant-derived anticancer drugs, vinca alkaloids, Texans, podo phyllo toxin, and camptothecins have all been employed in clinical settings. The literature on herbal treatments used to trigger apoptosis in cancer has been summarized in the current review.

3.13 NUCLEAR CONDENSATION BY DAPI METHOD

A hallmark of apoptotic cell death is nuclear condensation, which is characterised by altered chromatin structure and highly compacted, fragmented DNA. This is in contrast to necrotic cell death, in which the DNA is substantially unaltered but the chromatin structure is disturbed. The DAPI stain method, which is used to detect apoptosis, is characterised by the nucleus' compacted chromatin structure, which causes it to release a signal that apoptotic cells are present. Necrosis is occurring in healthy cells, although the chromatin structure is still distributed. A good tool for identifying apoptotic cells from healthy cells is the DAPI staining, which is frequently employed in apoptotic studies. Live cells are represented by the dark colours that emit the light one. The live cells are in that MMT's (Mitochondrial Membrane Test) one holosome, which is coloured black.

3.14 DAMODARAN et.al (2019)

Nuclear morphology and chromatin condensation signals are used in cancer detection for precise advanced stage classification. It is desirable to construct portable mobile microscopes to see nuclear and chromatin condensation patterns in clinical settings with limited infrastructure, even if such diagnostic procedures rely on high resolution imaging of the cell nucleus employing pricey microscopy devices. In this study, we create a movable fluorescent portable microscope that can capture high resolution images of the chromatin and nucleus. With similar accuracy to wide-field fluorescence microscopy, we were able to distinguish between normal and malignant cells using features based on nuclear morphometrics and chromatin roughness. Additionally, we were able to identify modest alterations in the nucleus and chromatin composition of cells that had undergone compressive stresses, cytoskeletal disruptions, and cytokines stimulation, their by highlighting the chromatin. DAPI is a dye that can be used as a tool to visualize nuclear changes and assess apoptosis. DAPI binds strongly and selectively to the minor groove of adenine-thymine regions of DNA. When bound to double-stranded DNA, DAPI absorbs light at 359 nm (ultraviolet light) and emits at 461 nm.

DAPI can stain dead cells, in fact it prefers to stain dead cells. This has to do with the membrane permeability of dead cells. It cannot stain live cells with intact membranes, unless used at high concentrations. DAPI is a fluorescent stain that binds strongly to A-T-rich regions in DNA. DAPI and PI only inefficiently pass through an intact cell membrane and, therefore, preferentially stain dead cells DAPI staining technique in this study reveals that, several nuclear alterations such as shrinked nucleus, condensed chromatin, nuclear breakage etc. were observed in all treated mice cells under fluorescence microscope where round and regular shape. Detection of apoptotic cells through DAPI staining. normal cells are round in shape and show less bright blue fluorescence. Whereas the apoptotic cells are brightly stained with the characteristic features of apoptosis such as chromatin condensation, nuclear fragmentation and aggregation of apoptotic bodies. normal cells, the blue fluorescence will be observed by fluorescent microscopy. With the process of apoptosis, the ability of permeability for dye is improved and the apoptotic cells will produce high blue fluorescence.

4.MATERIALS AND METHODS

- Sample
- Petri plate
- Conical flask
- Beaker
- Spatula
- Eppendrof tube
- Test tubes
- Magnetic Stirrer
- Distilled Water
- Water bath
- Centrifuge
- DMEM
- Antibiotics
- Cotton

* spectrometer
* shaker
*96 well plates
* cholesterol
* acridine orange
* propidium iodine
* DPPH
* Ethanol
* Chloroform
*Tween 80
* pipette
*Fetal Bovine Serum
*Trypsin
*Filter paper

METHODS

4.1. COLLECTION OF SAMPLES: -

We took the Kadukkai powder sample which is used in many health problems to cure the diseases. The plant has been shown to have numerous pharmacological and medicinal properties, including antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, radioprotective, cardioprotective, anti-arthritic, and gastrointestinal motility and apoptotic activity.

4.2 EXTRACTION OF ETHANOL AND WATER: -

We took the sample(10gm) mixed with 150 ml Distilled water and also mixed with 150 ml Ethanol and then we kept in shaker. Remove the solvents from the shaker and then filter both the solvents using filter paper and we done the phytochemical assays.

FIG4.2: -EXTRACT OF ETHANOL AND WATER

4.3 PHYTOCHEMICAL ASSAY: -

we performed phytochemical assays with the Ethanol and water solvent of plant extract 1 ml in each test tube and performed the following assay in both water and ethanol solvents by the following reagents.

4.3.1 Wagner's test: -

A few drops of Wagner's reagent are added to the ethanol and water plant extract solvent present in the test tube. Wagner's reagent is a mixture of iodine and potassium iodide in distilled water. This reagent gives the Reddish-brown precipitate if it is positive. It is used for detection of alkaloid.

4.3.2 Fehling's test: -

1ml of Fehling's solution A and solution B are added to the ethanol and water plant extract solvent present in the test tube. These test tubes are kept in the water bath for 20 mins. After cooling a red precipitate is indicate positive.

Fehling's solution A is a mixture of copper sulphate and distilled water whereas Fehling's solution B is a mixture of potassium sodium tartarated and sodium hydroxide in water. This test detects presence of sugar or carbohydrates.

4.3.3 Bontrager's test: -

A few drops of chloroform were added to the ethanol and water plant extract solvent present in the test tube. These test tubes are kept in the water bath for 20 mins. After cooling it was shaken and added with 1 ml of ammonia solution which precipitate pink colour that indicate positive. It is used to detect present of glycosides.

4.3.4 Foam test: -

The ethanol and water plant extract solvent present in the test tube is added with equal amount of distilled water, shaken vigorously and left and disturbed 2 to 5 mins. If the foam persist it indicates positive, It is used to detect saponin.

4.3.5 Biuret test: -

1 ml of 10% sodium hydroxide solution was treated with ethanol and water plant extract solvent present in the test tube and CuSO4 was added which precipitate violet colour. It is used to indicate the presence of protein and amino acids.

4.3.6 Ninhydrin test: -

2 drops of ninhydrin solution is added to water and ethanol plant extract solvent present in the test tube that indicates purple colour is the detection of amino acids

4.3.7 Ferric chloride test: -

The ethanol and water plant extract solvent present in the test tube is added with 5ml of distilled water. To these few drops of neutral 5% of Fecl2 solution was added that precipitate green colour is the indication of phenolic compounds.

4.3.8 Detection of flavonoid compounds: -

3 ml of dilute ammonia was added to water and ethanol plant extract solvent present in the test tube. It was followed by addition of 1 ml of concentrated H2SO4 was carefully added to form a yellow coloration.

4.3.9 Test for terpenoids: -

2 ml of chloroform was added to water and ethanol plant extract solvent present in the test tube and mixed well. This was added with concentrated H2SO4 was added to form a reddish-brown layer.

4.3.10 Detection of steroids: -

2micro liter of chloroform was added to water and ethanol plant extract solvent present in the test tube and a few drops of acetic anhydride were poured followed by concentrated H2SO4. A mixture of blue and green colour shows presence of steroids.

4.3.11 Iodine test: -

Water and ethanol plant extract solvent present in the test tube was treated with 1%of iodine solution. Appearance of transient red colour indicates the presence of tannins.

4.4 ANTIOXIDANT ASSAY BY DPPH METHOD: -

Six test tubes were taken and named as control and other five tubes as 1 to 5. From 1 to 5 test tubes are added with water and ethanol plant extract solvent with the ratio of 20µl to 100µl

To each test tube 2ml of DPPH (2,2 diphenyl-1-picrylhydrapicrylhydrazyl) solution was added including the control test tubes. After adding the DPPH solution, all the test tubes are kept in dark room for incubation for 20 mins. After this it was carried out using calorimeter. A blank was set using methanol at 570nm. This result was taken and values are obtained.

4.5 SYNTHESIS OF NIOSOMES: -

We took 100 ml of distilled water in conical flask mixed with Tween 80 (1.31 ml) and kept it in stirrer to mix well for 1 hour, then 100ml of chloroform is mixed with 0.2 gm of cholesterol.

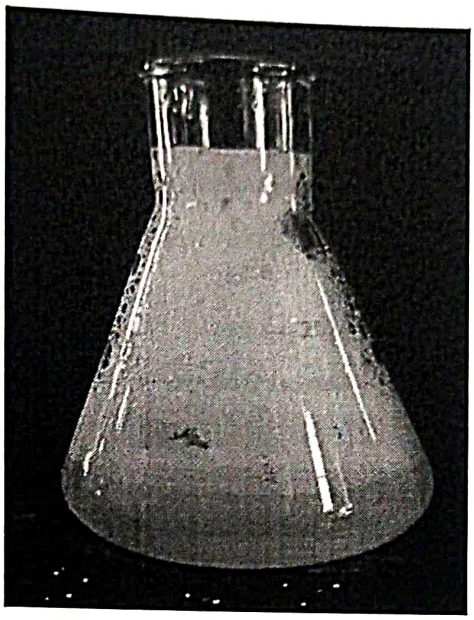


FIG4.5.1: -TWEEN 80+CHLOROFORM

Both the liquid mixed well and put it in stirrer and heat the liquid at 40C for 2 hours and kept in the refrigerator.

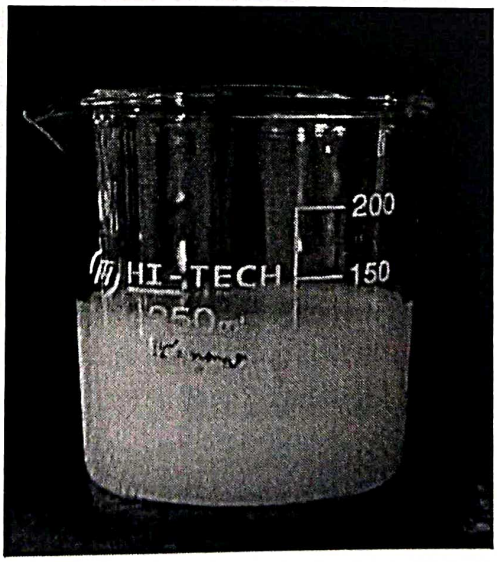


FIG4.5.2: - NIOSOMES

4.6 CHARACTERIZATION OF NIOSOME BY UV VISIBLE AND FTIR :-

It is very important to characterize their physical and chemical properties, such as their size, distribution, composition and electronic structure. This technique is based on the measurement of the absorption or transmission of light by a sample in the UV-Visible region of the electromagnetic spectrum. The wavelength is set with a range of 200 to 800nm and these are seen in graph.

4.7 NATURAL DRUG INCORPORATED WITH NIOSOME: -

We took the niosome gel and kept in centrifuge for 15 min at 8000 rpm. After centrifuge we took the pellet in the beaker added and it is added with niosomes and ethanol extract and kept in stirrer for 2 hours at 60c.

FIG4.7: - CHOLESTROL+ETHANOL EXTRACT

4.8 STUDY ANTIOXIDANT ACTIVITY OF NIOSOMES: -

Now we took the solvent from the stirrer and performed antioxidant assay by taking 6 beakers and named as control,1,2,3,4,5. These 1 to 5 test tubes are added with ethanol and niosome gel .2ml of DPPH in each test tube and seen the following values.

4..9INVITRO CYTOTOXICITY OF NIOSOMES: -

4.9.1 Cell Culture Maintenance

Vero (African green monkey kidney normal epithelial cell line) and PA-1 (Human ovarian teratocarcinoma epithelial cell line) were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin. They were maintained at 37°C with 5% CO_2 in 95% air humidified incubator.

4.9.2 Cytotoxic effect

The cytotoxic effect of the sample was tested against Vero and PA-1 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The cells were seeded in 96-well microplates (1 x 10^6 cells/well) and incubated at 37°C for 48 h in 5% CO_2 incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of sample 20,40,60,80,100 µl and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µl of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37°C in the dark for 2 h. nm The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrometrically at 570NM. Percentage of cell viability was calculated using the formula,

Cell viability (%) = (Absorbance of sample/Absorbance of control) X10

TABLE4.9.2.1: -VERO CELL LINE

Concentrations (µg/mL)	Absorbance		Average	Cell Viability (%)
	I	II		
Control	0.746	0.734	0.74	100
20	0.723	0.716	0.7195	97.22972973
40	0.696	0.691	0.6935	93.71621622
60	0.667	0.655	0.661	89.32432432
80	0.629	0.617	0.623	84.18918919
100	0.587	0.578	0.5825	78.71621622

TABLE4.9.2.2: - PA-1CELL LINE

Concentrations (µg/mL)	Absorbance		Average	Cell Viability (%)
	I	II		
Control	0.943	0.931	0.937	100
20	0.764	0.758	0.761	81.21664888
40	0.597	0.591	0.594	63.39381003
60	0.387	0.368	0.3775	40.28815368
80	0.216	0.224	0.22	23.4791889
100	0.139	0.133	0.136	14.51440768

4.10 ANTICANCER ANALYSIS: -

The effect of noisome is to growth of ovarian cancer cells was evaluated at different concentrations and time points. Ovarian cancer cells were treated with various concentrations 20,40,60,80,100µl of niosome for 24 hours.

The Results showed that the niosomes is inhibited the growth of ovarian cancer cells in a dose defended manner. After 24 hours the highest concentration of 20 µl showed the greatest inhibition with the cell viability of 97.22 % compared to the other group. Similarly, at 48 hours the highest concentration of 20µl showed the greatest inhibition with the cell viability of 81.21% compared to the other group.

The study also reported that the niosomes are induced cell apoptosis in a dose dependent manner.

4.11 APOPTOSIS ANALYSIS: -

An apoptosis analysis experiment involves several steps including cell culture treatment with apoptosis and analysis of the cells. These cells were exposed to our treatment was performed for 24 hours and 48 hours concentrations of 20 to 100µl were used to determine the optimal condition for inducing apoptosis.

During the treatment the treatment the cells were collected by trypsinization and washed them with PBS.

4.11.1 Staining

Acridine Orange staining is the common technique used in apoptosis analysis.

This fluorescent dye that can intercalate with DNA and RNA and emit Green Fluorescent. The stained cells are then examined under a fluorescent microscope to the number of live cells and apoptotic cells were counted whereas the Propidium iodide are commonly used Dye is distinguished between the early and late Apoptosis. The cells were incubated with PI for 15 min at room temperature and kept in the dark. Mostly the live cells are observed in green fluorescence and dead cells are observed in red fluorescence. Dark red is observed in necrosis.

4.12 CHROMATIN CONDENSATION BY DAPI METHOD: -

The cells are washed with Phosphate-buffered Saline (PBS) to remove excess DAPI. The cells are grown ovarian cancer cells in culture and treated with the experimental conditions to induce apoptosis. Observed cells under an Inverted Microscope with appropriate filters to detect DAPI-STAIN. The DAPI method is highly specific and sensitive for detecting apoptotic cells based on their patterns.

5.OBSERVATION AND RESULTS

5.1 PHYTOCHEMICAL ASSAY: -

The analysis of ethanolic plant extract sample revealed the presence of alkaloids, carbohydrates, proteins, phenolic compounds, flavonoid and terpenoids. The analysis of water plant extract sample revealed the presence of carbohydrates, phenolic compounds, flavonoid.

Alkaloids are the compounds that contain antimicrobial activities, carbohydrates are the essential compounds found in all living organism which provide energy to the body. Phenolic compounds are believed to play vital role in preventing various chronic diseases. Flavonoids are found in plants and have various Anti-oxidant activities whereas the Terpenoids exhibit antibacterial activity.

The results of ethanolic and water plant extract samples are done by phytochemical assay are given below: -

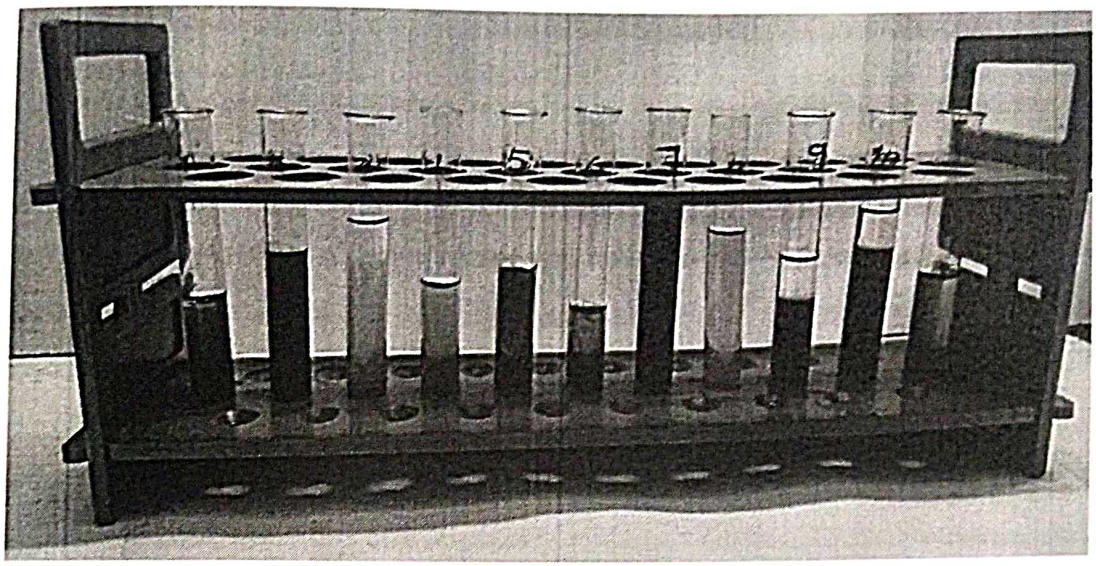


Fig5.1.1: - PHYTOCHEMICAL ASSAY OF ETHANOL EXTRACT

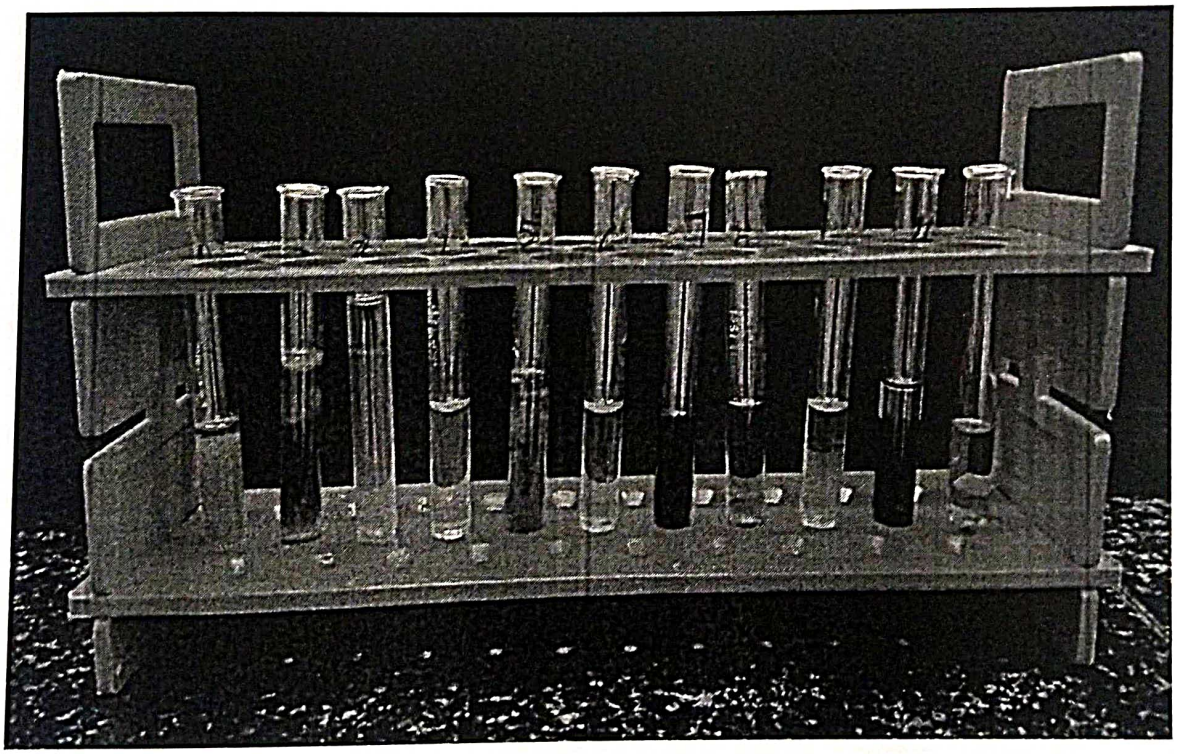


Fig5.1.2: - PHYTOCHEMICAL ASSAY OF WATER EXTRACT

NAME OF THE TEST	REAGENTS	CHANGE OF COLORS	ETHANOL EXTRACT (Yes/No)	WATER EXTRACT (yes/No)
Alkaloid Test	Wagner's test	Presence of reddish-brown precipitate	YES	NO
Carbohydrate test	Fehling's solution 1 and 2	Presence of red precipitate	YES	YES
Glycosides test	Conc. HCL	Presence of pink color	NO	NO
Saponin test	Distilled water	Foam persistent	YES	NO
Protein test	2% cuso4 ,95% c2h6o, potassium hydroxide	Purplish violet color	YES	NO
Amino acid test	Ninhydrin test	Purple/pink color	NO	NO
Phenolic compounds test	5% ferric chloride	Dark green color	YES	YES
Flavonoid test	Dilute ammonia, conc. HCL	Yellow Coloration precipitate	YES	YES
Terpenoid test	Chloroform, conc. H2so4	Reddish-Brown layer	YES	NO
Sterroid test	Chloroform, Acetic Anhydride, conc H2SO4	Blue and Green coloration	NO	NO
Tannins test	1% Iodine solution	Transient red color	NO	

TABLE5.1: -PHYTOCHEMICAL ASSAY OF ETHANOL AND WATER EXTRACT

5.2 ANTIOXIDANT ASSAY BY DPPH METHOD: -

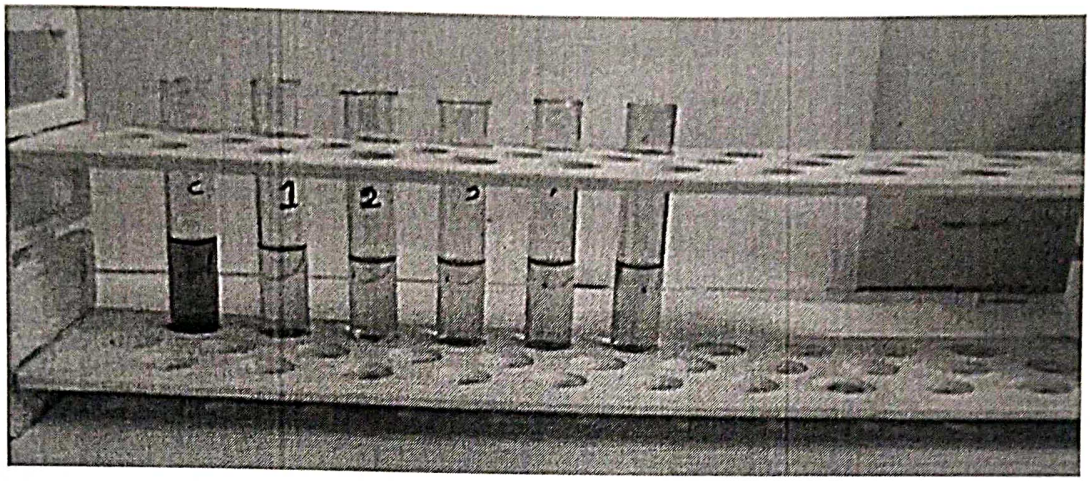


FIG 5.2.1: - ANTIOXIDANT ASSAY OF ETHANOL EXTRACT

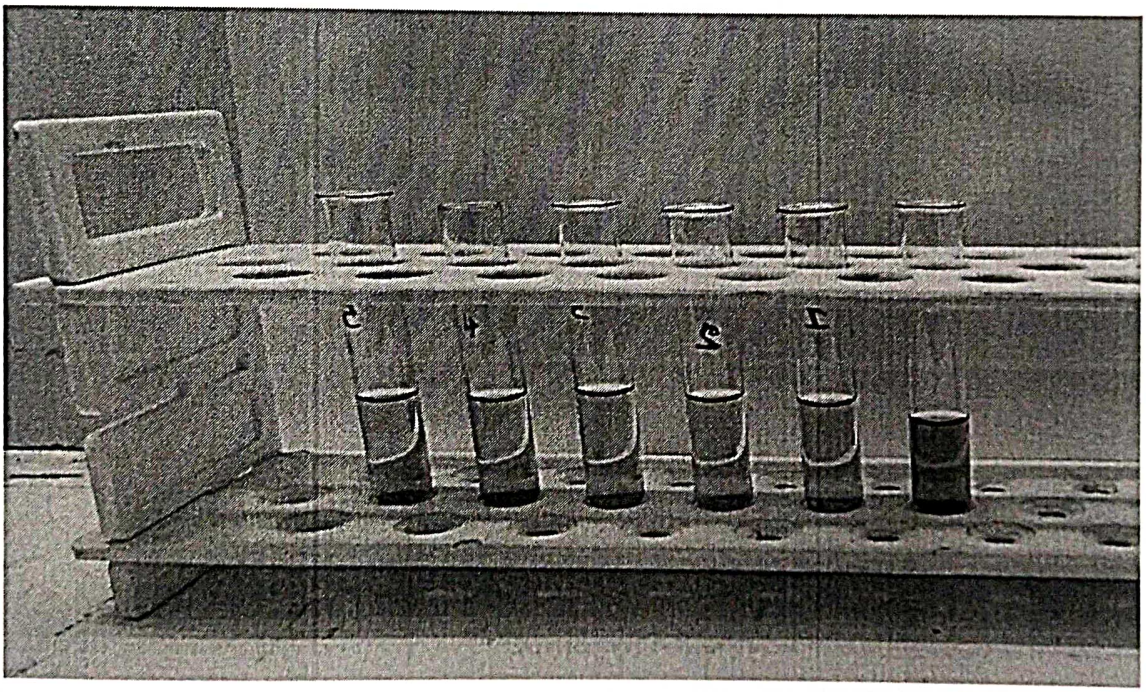
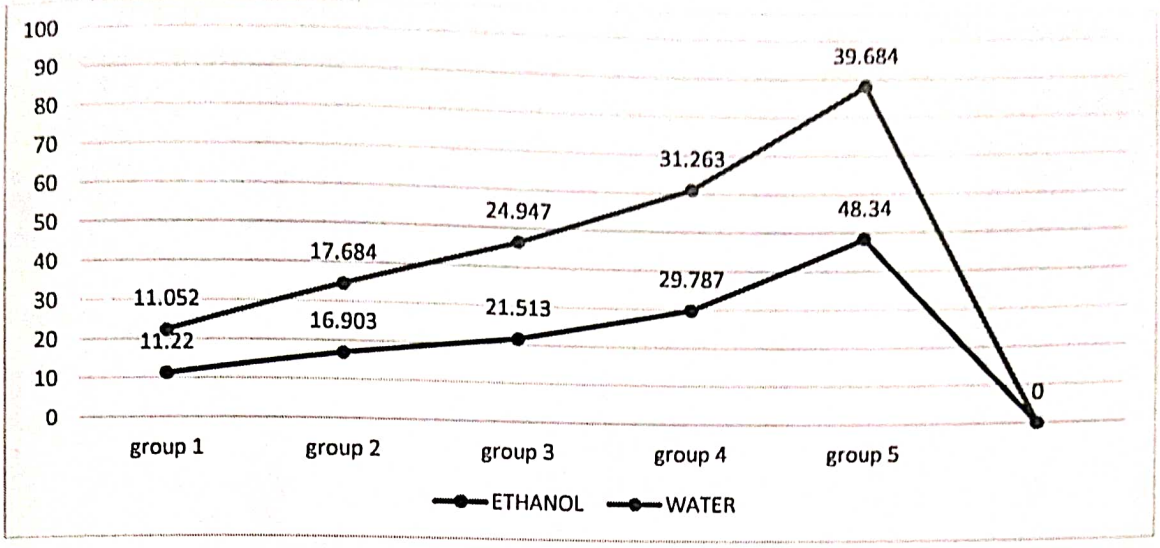


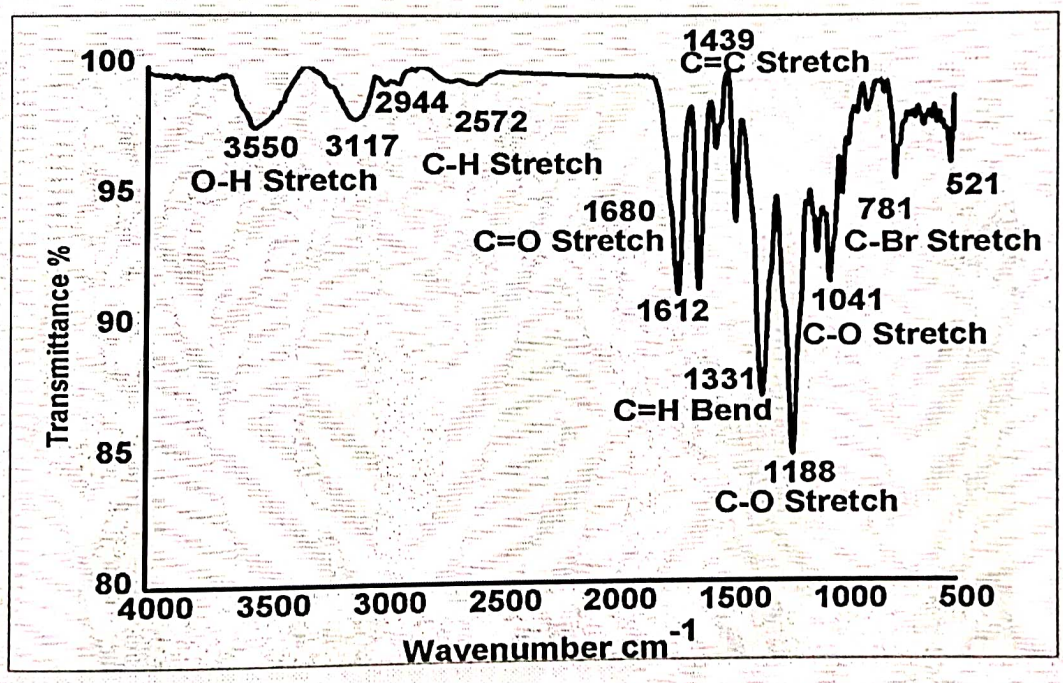
FIG 5.2.2: - ANTIOXIDANT ASSAY OF WATER EXTRACT



GRAPH 5.2: - ANTIOXIDANT ASSAY OF ETHANOL AND WATER SOLVENT

Water is more effective than ethanol in the above graph.

5.3: - FTIR ANALYSIS: -



Third-generation infrared spectrometers, or FTIR spectrometers, memories the fundamental absorption values of various compounds in order to determine the functional group of the chemical. We have only used infrared absorption spectroscopy at a wave number .

5.4 STUDY ANTIOXIDANT ACTIVITY OF NIOSOMES: -

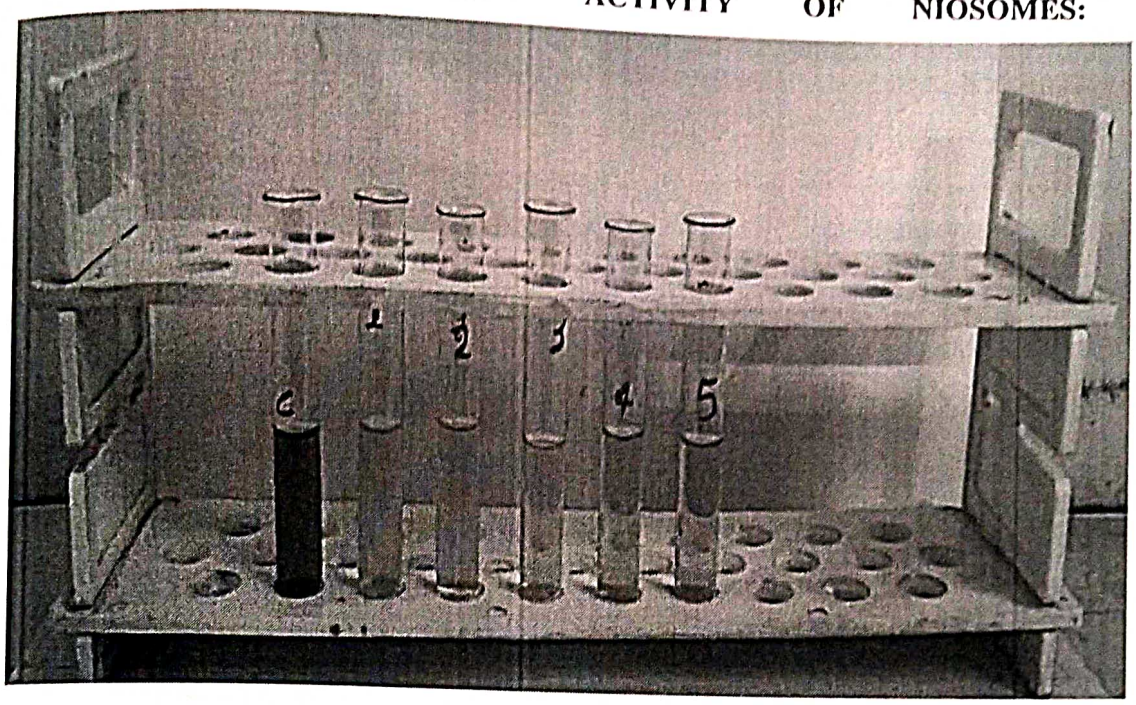
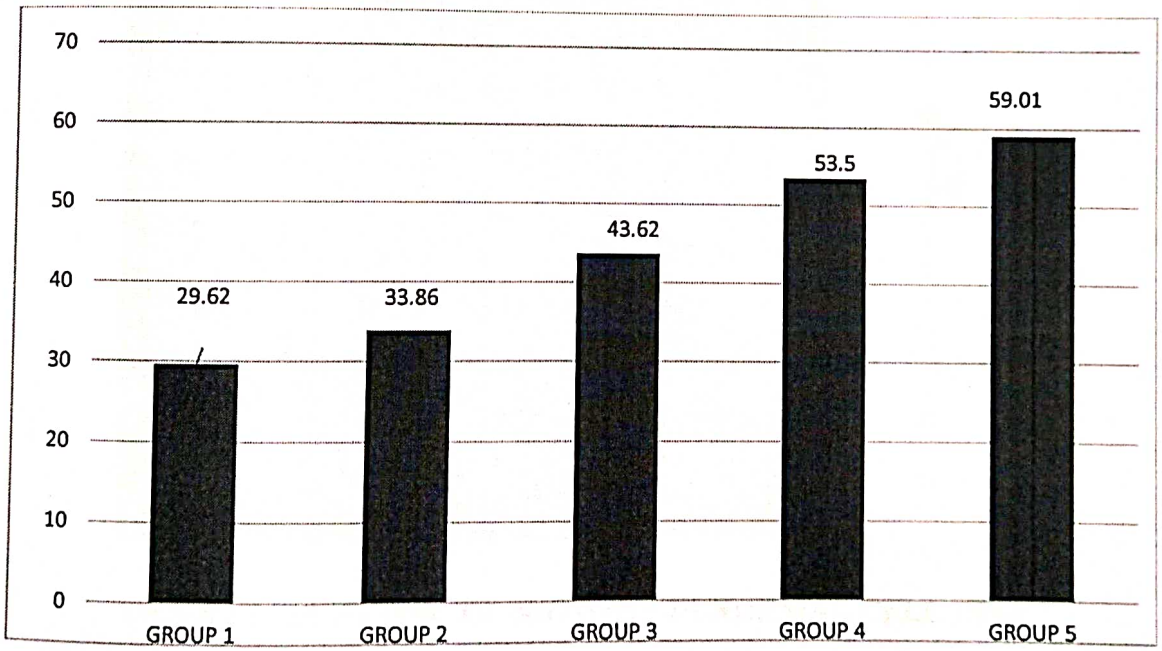


FIG 5.4.1: - ANTIOXIDANT ASSAY BY NIOSOMES



GRAPH 5.4.2: -ANTIOXIDANT ASSAY OF NIOSOMES

5.5 INVITRO CYTOTOXICITY OF NIOSOMES: -

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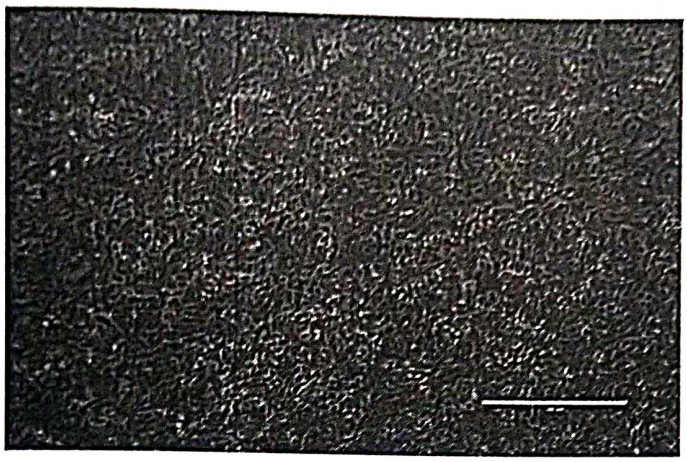

FIG5.5.1: -CONTROL OF NORMAL CELL LINE

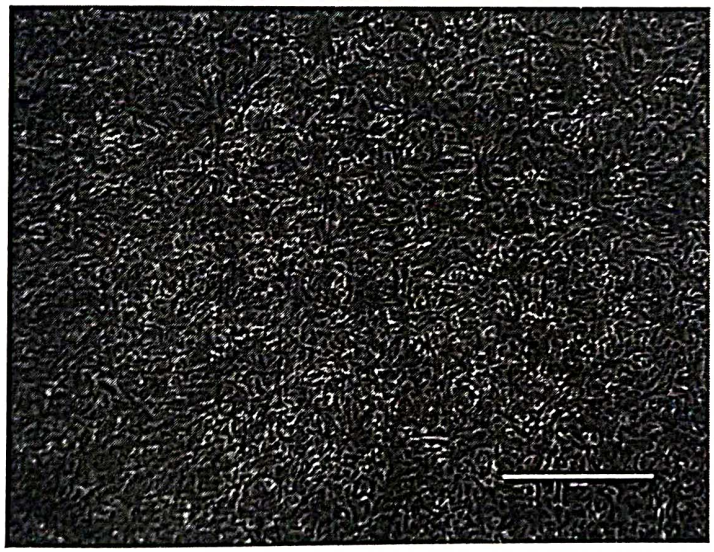

FIG5.5.2 : - 20 µl CONC OF NIOSOME IN NORMAL CELL LINE

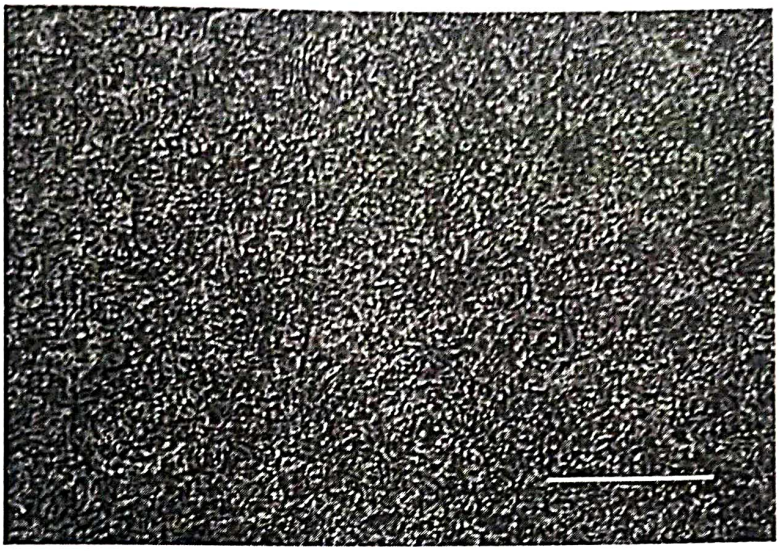

FIG 5.5.3: 40µl CONC OF NIOSOME IN NORMAL CELL LINE

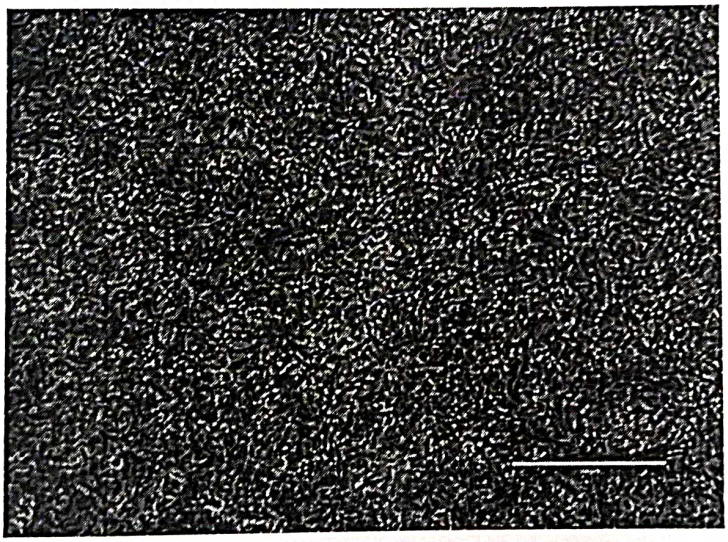

FIG 5.5.4: - 60µl CONC OF NIOSOME IN NORMAL CELL LINE

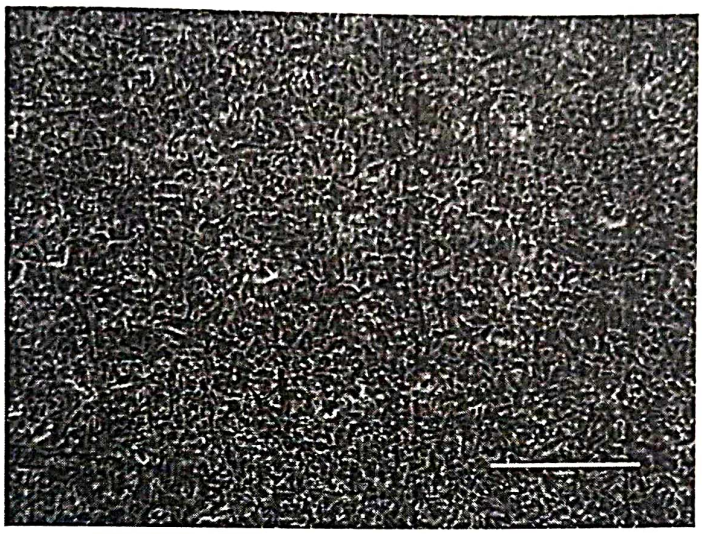

FIG5.5.5: - 80µl CONC OF NIOSOME IN NORMAL CELL LINE

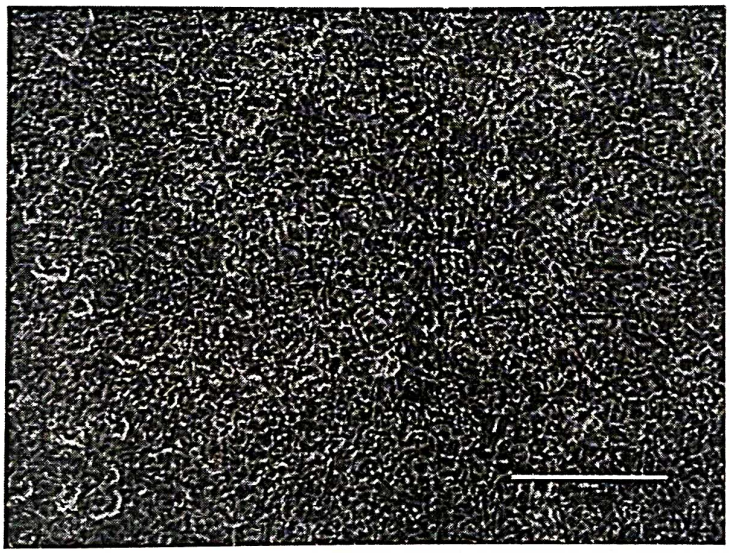

FIG5.5.6: - 100µl CONC OF NIOSOME IN NORMAL CELL LINE

5.6 ANTICANCER ANALYSIS: -

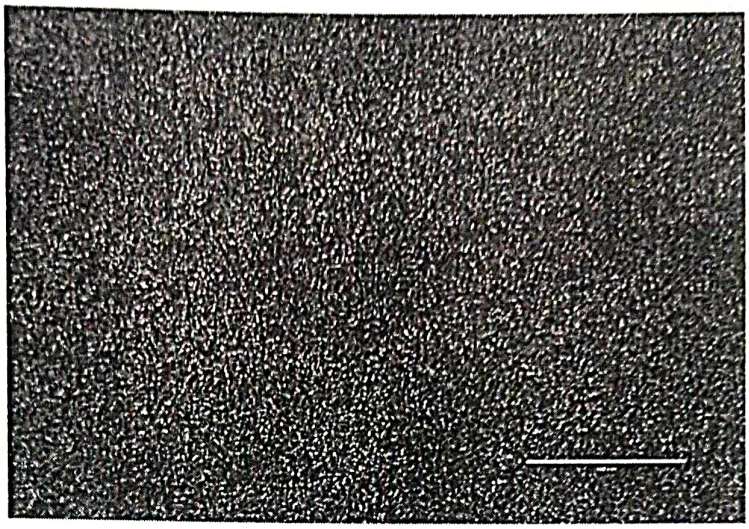

FIG 5.6.1: -CONTROL OF OVARIAN CANCER CELL LINE

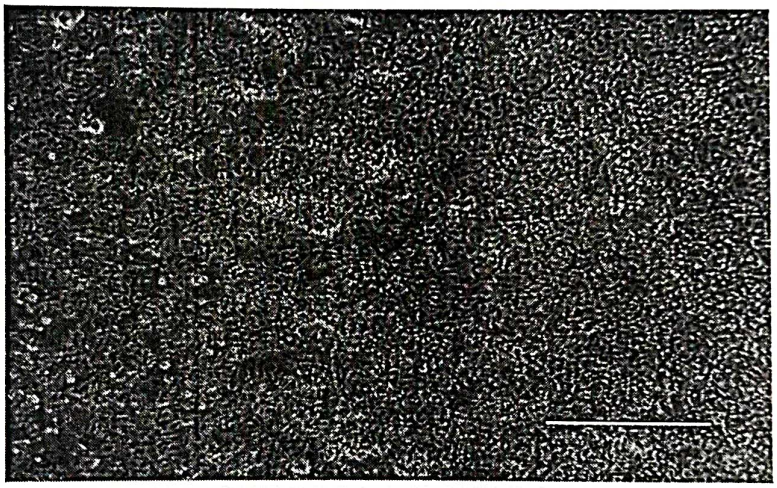

FIG 5.6.2: - 20µl CONC OF OVARIAN IN CANCER CELL LINE

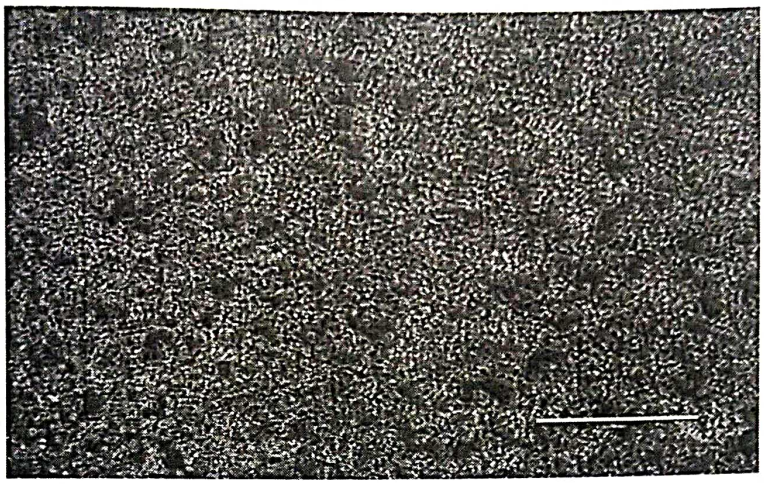

FIG 5.6.3: - 40µl CONC OF OVARIAN IN CANCER CELL LINE

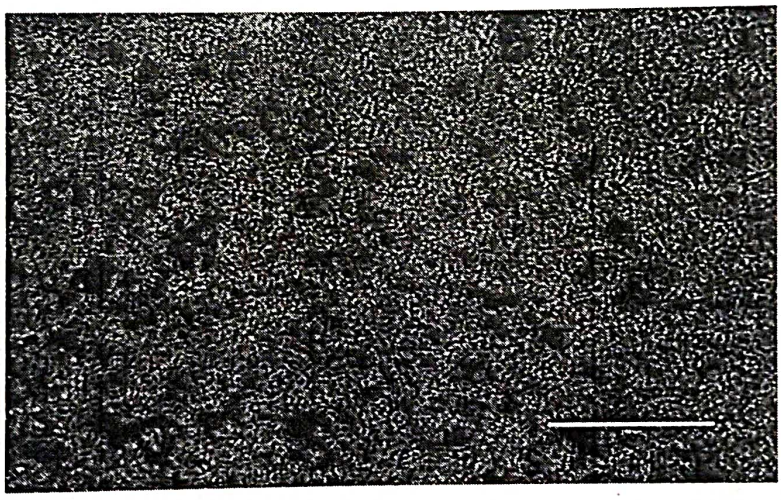

FIG5.6.4: - 60µl CONC OF OVARIAN IN CANCER CELL LINE

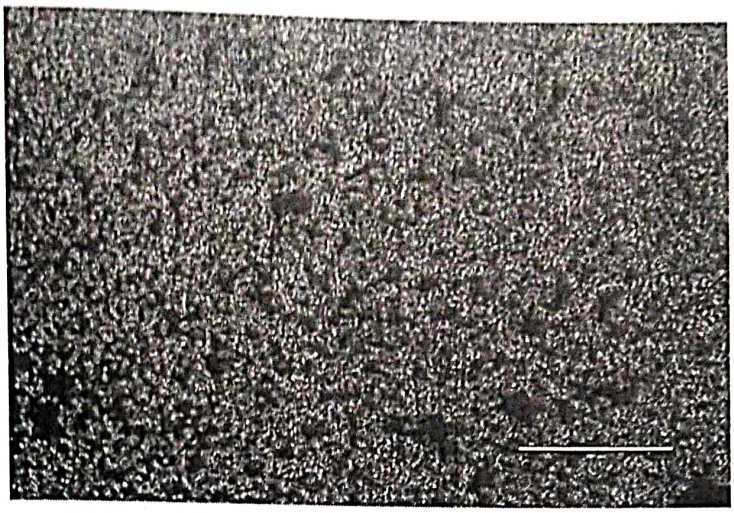

FIG5.6.5: - 80µl CONC OF OVARIAN IN CANCER CELL LINE

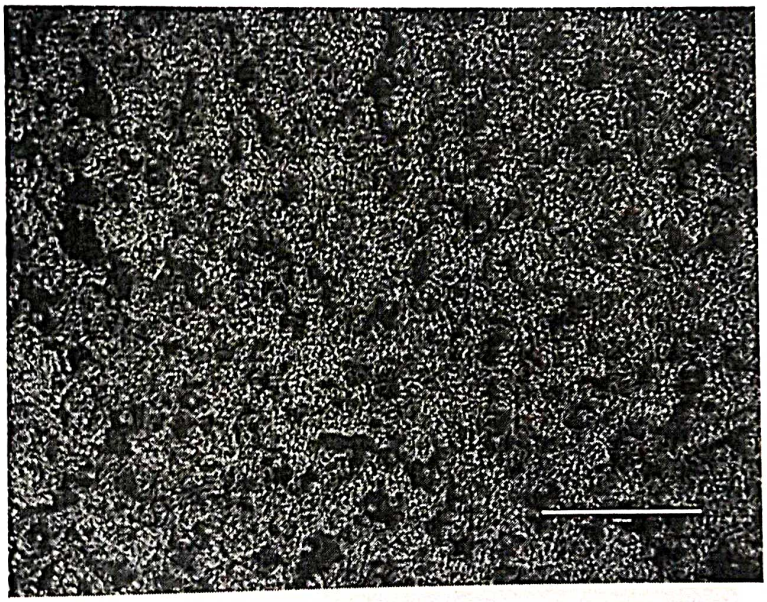

FIG5.6.6: - 100µl CONC OF OVARIAN IN CANCER CELL LINE

5.7 APOPTOSIS ANALYSIS: -

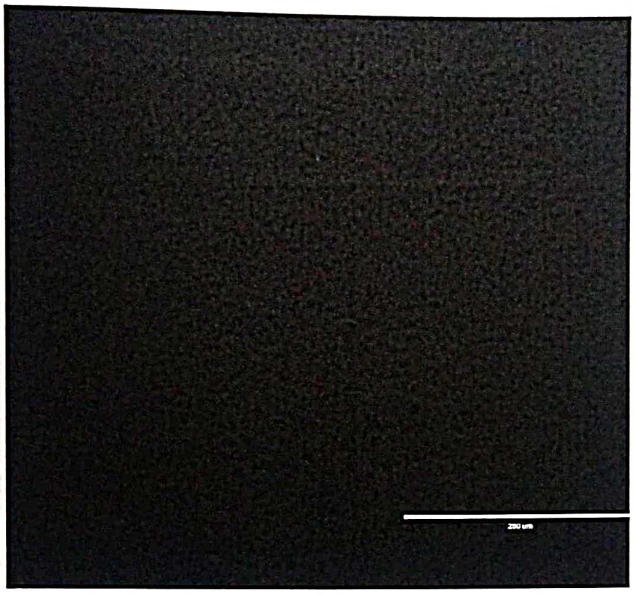

FIG 5.7.1: - CONTROL CELLS STAINED WITH PI

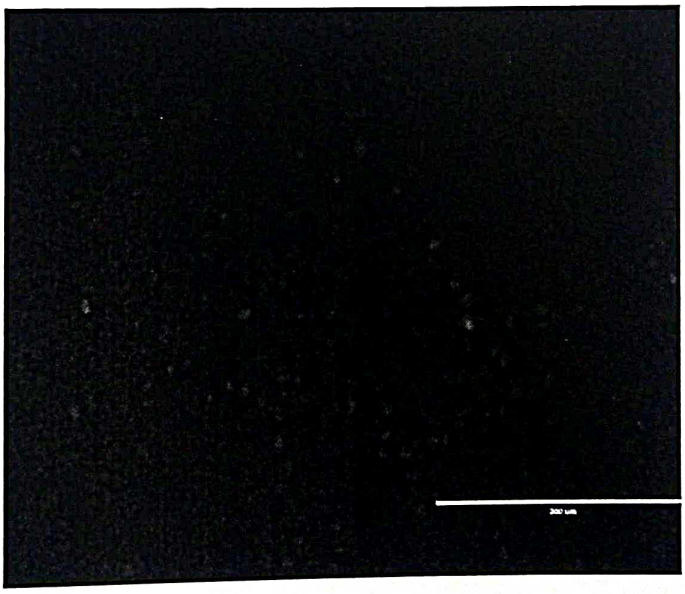

FIG 5.7.2: - TREATED CELLS STAINED WITH PI

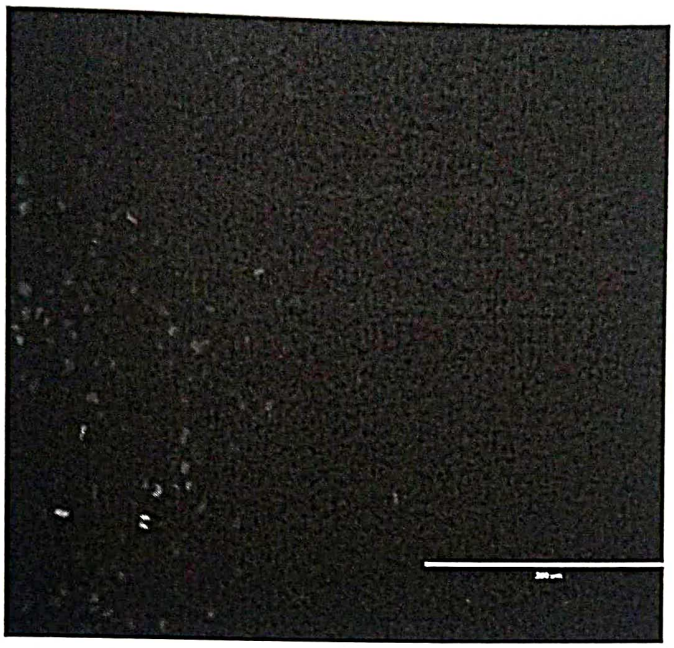

FIG 5.7.3: - CONTROL CELLS STAINED WITH AO

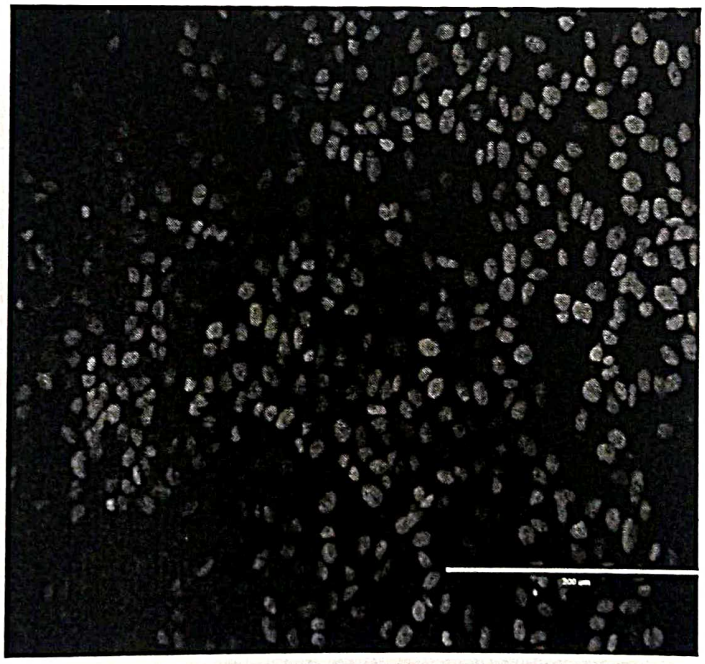

FIG 5.7.4: - TREATED CELLS STAINED WITH AO

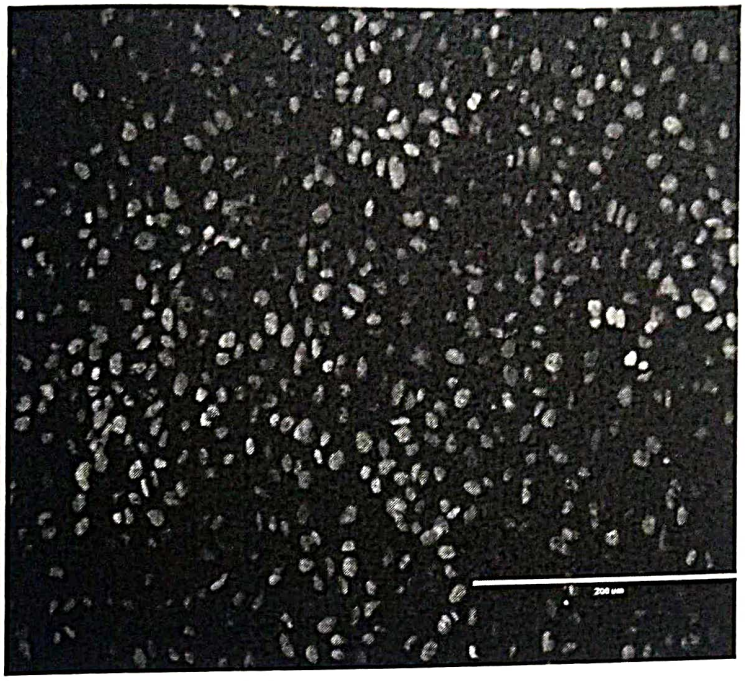

FIG 5.7.5: - CONTROL AND TREATED CELLS STAINED WITH AO& PI

5.8 CHROMATIN CONDENSATION BY DAPI METHOD: -

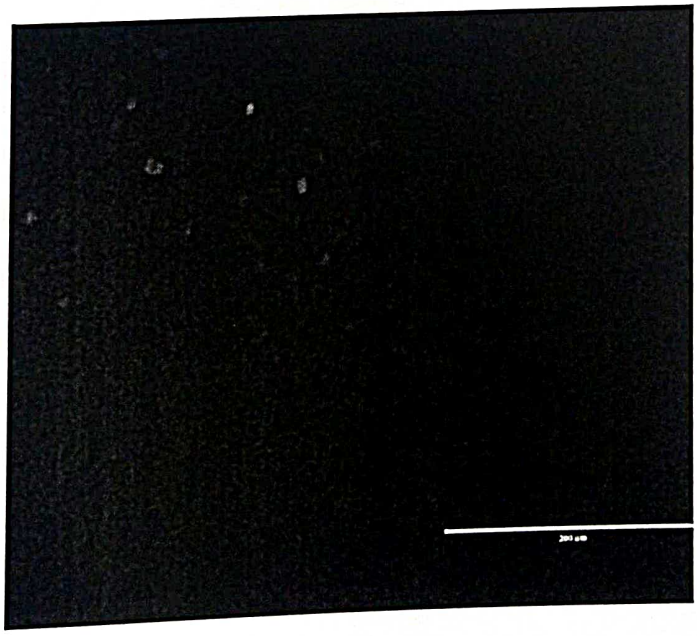

FIG 5.8.1: - CONTROL CELLS STAINED WITH DAPI

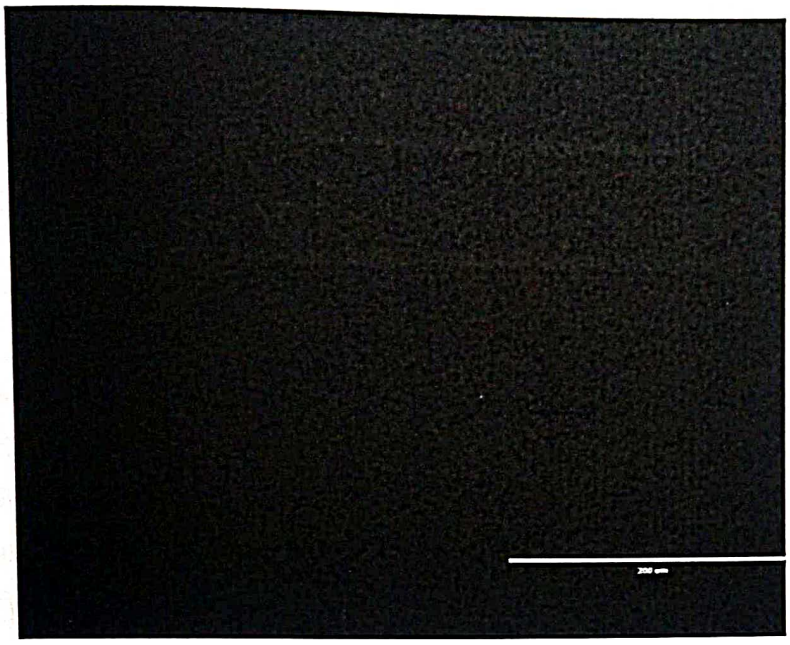

FIG: 5.8.2 - TREATED CELLS STAINED WITH DAPI

6.CONCLUSION

Niosomes is a brand-new and effective method of medication delivery. Numerous medications can be encapsulated into niosomes by adding the proper nonionic surfactant and cholesterol to the vesicular membrane. The result of the study shows that this is used in next generation on cancer treatment through modern therapy using natural drug embedded through niosome receptors.

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