A DISSERTATION ON

EVALUATION OF ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF *CIMICIFUGA RACEMOSA* RHIZOME EXTRACT AGAINST CERVICAL CANCER HeLa CELLS

SUBMITTED TO THE

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DEGREE OF MASTER OF SCIENCE IN

BIOTECHNOLOGY

BY

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ENROLMENT NUMBER: 2000102721

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Kitur Chepngeno Zibiah** during the month of February to June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by the candidate herself. The thesis is entitled "**Evaluation of antioxidant and cytotoxic potential of *Cimicifuga racemosa* rhizome extract against cervical cancer HeLa cells"** is therefore, being forwarded for acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow, (U.P).

Dr. Irfan Ahmad Ansari
Associate Professor
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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Kitur Chepngeno Zibiah** student of MSc. Biotechnology (IVth semester), Integral University has completed her 4 months dissertation work entitled "**Evaluation of antioxidant and cytotoxic potential of *Cimicifuga racemosa* rhizome extract against cervical cancer HeLa cells"** successfully. She did this work from February-June under the guidance of Dr. Irfan Ahmad Ansari. The dissertation was a compulsory part of her MSc. degree.

I wish her good luck and bright future.

Dr. Snober S. Mir
HOD
(Department of Biosciences)

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Kitur Chepngeno Zibiah

CONTENT

LIST OF ABBREVIATIONS

CC	Cervical Cancer
CR	*Cimicifuga recemosa*
HR HPV	High Risk Human Papillomavirus
APC	Adenomatous polyposis coli
COX	Cyclooxygenase
EDTA	Ethylene diamine tetraacetatic acid
Hh	Hedgehog
JAG1	Jagged 1
MTT	(3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide)
PBS	Phosphate buffer saline
Ptch1	Patched1
TRAIL	TNF-related apoptosis-inducing ligand
FADD	Fas-associated death domain protein
TRADD	TNF receptor-associated death domain
TRAF2	TNF receptor-associated factor-2
VEGF	Vascular endothelial growth factor
WHO	World health organization
WNT	Wingless-related integration site

LIST OF FIGURES

ABSTRACT

The rhizome of *Cimicifuga racemosa* has been in use as traditional medicine for long periods because of its active compounds. It has been utilized in various models to provide medicinal benefits to patients who are suffering from breast cancer, prostate cancer, diarrhea, arthritis and muscle pain. Its rhizome has multiple secondary metabolites that successfully deliver therapeutic effects for example antioxidants, triterpenoids, phenolic compounds, amides, glycosides, alkaloids, tannins, flavonoids and steroids.

Given that the conventional cancer treatments such as the use of synthetic drugs that are commonly utilized in hospitals have been reported to have undesirable side effects, plant derived cancer drugs have been adopted worldwide because they have less side effects to human cells. The purpose of this project was to determine the cytotoxicity of *Cimicifuga racemosa* rhizome extract on cervical cancer HeLa cells.

The extract of *Cimicifuga racemosa* rhizome was analyzed for its antioxidant activity using FRAP Assay and the results were standardized using Ascorbic acid. *Cimicifuga racemosa* rhizome extract proved to have significant amounts of antioxidants. The HeLa cells were treated with different concentration of the rhizome extract for 24 h and it showed significant morphological effect as the concentration was increased successively.

The MTT Assay performed on the HeLa cells that had been treated with different concentrations of the rhizome extract for 24 h showed reduced cell viability successively per well as the extract concentration increased. The MTT Assay results was used to generate $IC_{50\ value}$ and *Cimicifuga racemosa* rhizome extract exhibited its half of maximal inhibitory effect on HeLa cells at 297.6 ±5.39 µg/ml at 24 h. This value displayed the efficiency of the extract in reducing the viability of HeLa cells.

This study confirmed the potential of *Cimicifuga racemosa* rhizome extract as a chemo-preventive agent in human cervical cancer HeLa cells with the involvement of apoptosis.

INTRODUCTION

Cancer, also termed as malignancy is a disease where body's cells grow and spread uncontrollably over other parts of the body (Wang *et al*., 2018). This condition develops due to multipart interaction of genetic and naturally occurring factors such as chemical, biological and physical carcinogens. The carcinogens have the ability to coordinate carcinogenesis in the normal cells and lead to malignancy (Moore *et al.,* 2017). During the life cycle of a human being, normal cells undergo division to replace themselves. Typically, the process of cell division is under a tight control mechanism that ensures the fidelity of the system. However, due to carcinogenesis, normal cells lose sensitivity to the cell cycle control mechanism and begin dividing and growing abnormally (Matthews *et al*., 2022).

Cancer as a disease is designated by distractions in typical cellular functions. This is brought about by the mutagenic events as a result of carcinogens that disrupts a given cell's genetic material and as a result lead to deregulation of the signaling pathways that are in control of the cell's essential processes such as regulation of cell division, cell differentiation, DNA repair and cell death (Liakos *et al.,* 2017). As soon as these pathways are altered, cancer cells become competent to divide, grow uncontrollably and break loose to move to infect other parts of the body. Lung, prostate, colorectal, skin and liver cancer are the common kinds that affect men on the other hand, breast, endometrial, cervical and ovarian cancer most often affect women (WHO, 2018).

Given that cancer develops in a complex manner, there are a number of characteristics it has to possess to be able to initiate and sustain its growth in any part of the body (Hoarau-Véchot *et al.,* 2018). For this reason, the characteristics are well known as the ten hallmarks of cancer. They include; self-sufficiency in growth signals, evading growth-inhibitory signals, avoiding programmed cell death, permitting limitless replication**,** sustained angiogenesis, initiation of invasion and metastasis**,** evading immune destruction, deregulation of cellular metabolism**,** genome instability and mutation and tumor facilitating inflammation. Due to these hallmarks, cancer is a major health issue and it is among the utmost fatal diseases that devastate the worldwide population for

instance; in 2020, it was the leading cause of death globally, accounting to approximately 10 million deaths (WHO, 2022).

Cervical cancer is the second most occurring cancer in women worldwide. In 2020, it contributed to nearly 604,000 new cancer cases and 342,000 deaths. Its effect is majorly experienced by women in developing countries. Molecular diagnosis has given clear evidence that this disease is linked to certain strains of human papillomavirus (HPV). There are more than 100 types of HPV and close to 40 strains have the capacity to infect the genital tract.

More often, this disease is prevalent in women who are thirty years and above. Its main cause being linked to prolonged HPV infection which have HPV 16 and 18 strains. HPV is a viral infection that causes growths in different parts of the body depending on the strain (Al-Eitan *et al.,* 2020). Given that for cervical cancer to develop there is need for prolonged HPV infection, the high mortality rate can easily be reduced if the cancer is detected early and treated effectively. This calls for sensitization of women to go for screening regularly. Furthermore, currently HPV vaccines that are efficient in killing the strains that can cause cervical cancer are available for all women regardless of their age (Kim *et al.,* 2018). With the forementioned approaches, it is possible to mitigate the risk of losing several women to cervical cancer.

In Kenya, cervical cancer is the second most occurring cancer in women after breast cancer. As at 2020, the global cancer observatory through the international agency for Research on Cancer (IARC) highlighted 5,236 new cervical cancer cases in Kenya. This value cumulated to 19.7% of the new infection among all Kenyan women in all age groups. Regrettably, cervical cancer caused 3,211 deaths in Kenya in 2020 and in 2021, this type of disease registered more deaths than any other type of cancer. The prevalence of this disease among Kenyan women across all ages was at 10,881 as at 2020. According to WHO, the high death rates of cervical cancer in developing nations is as a result of limited health care services, low scope of public awareness, absence of screening programs as well as scarce treatment plans.

Cervical cancers frequently arise in persons who fail to seek medical attention when they have HPV infection (Fontham *et al*., 2020). In that way, they host oncogene expression for several years. In most instances, HPV clears on its own from the human system but this is not the case when it comes to particular HPV strains that have the capacity to initiate cancer. Immune deterioration more commonly results in the viral clearance and in other situations it causes the virus to be maintained in symptomless state in the basal cells (McBride *et al*., 2022). During the inception of this disease, there are naturally no symptoms. Afterwards, when the HPV has transformed the normal cells and made them oncogenic (Gupta *et al.,* 2018), symptoms which vary at length may begin to show. These include; irregular vaginal bleeding, pain in the pelvic and pain during sexual intercourse. Other risk factors that have capacity to contribute to development of cervical cancer range from smoking, weakened immune system, long term use of birth control pills, and having many sexual partners.

Multiple signaling pathways such as Wnt, Notch, Hedgehog and apoptosis have been identified by researchers to have undergone some levels of deregulation in cervical cancer (Yang *et al.,* 2020). This goes a long way to influence the existence of characteristics that are portrayed by this disease.

For cervical cancer treatment to be effective the extent of disease spread comes in play (Šarenac *et al.,* 2019). This is because when the cancer is still resident in a particular tissue it is much easier to target its location other than when it has spread to various tissues (Mami-Chouaib *et al.,* 2018). Given that cancer treatments are regularly complex, hospitals have adapted to multidisciplinary teams (MDTs) to try to counter the cervical cancer by modifying treatment programs for every patient (Wait *et al.,* 2017). Commonly cervical cancer treatments range from surgery, radiation therapy, chemotherapy, targeted therapy as well as immunotherapy. When the disease has spread, a number of treatments are combined to boost the chances of killing cervical cancer cells (Ferrall *et al.,* 2021).

PROBLEM STATEMENT

The conventional cancer treatments such as the use of synthetic drugs that are commonly utilized in hospitals are expensive for patients and they have been reported to have undesirable side effects (Glynn & Bhikha *et al.,* 2019). For this reason, plant derived cancer drugs (Iqbal *et al.,* 2017) have been adopted worldwide for example the use of Vincristine, vinblastine, paclitaxel, black cohosh and ADS among others.

JUSTIFICATION

Drugs derived from natural sources do not have major side effects to human cells (Roy *et al*., 2018) as compared to conventional ones. Nevertheless, they have active ingredients that have therapeutic effect (Kooti *et al.,* 2017).

Based on research, the rhizome of *Cimicifuga racemosa* extract has been proved to have multiple secondary metabolites which when ingested get absorbed in the blood stream and are maintained at certain levels in given target organs for a particular time and thus successfully deliver therapeutic effects. Its bioactive components include; triterpenoids, phenolic compounds, amides, glycosides, alkaloids, tannins, flavonoids and steroids.

The presence of the active compounds has made it possible for *Cimicifuga racemosa* rhizome extract to be used in various models to provide medicinal benefits to patients who are suffering from breast cancer, prostate cancer, diarrhea, arthritis and muscle pain (Guo et al., 2017). Such bioactive ingredients have the potential to cause anti-inflammation, anti-oxidation, antitumor and neuroprotection.

In this paper we evaluate the antioxidant activity and cytotoxicity effect of *Cimicifuga racemosa* rhizome extract on cervical cancer HeLa cell lines.

Objectives

Main Objective:

To investigate the efficacy of *Cimicifuga racemosa* rhizome extract against cervical cancer HeLa cells.

Specific Objectives:

1. Extraction of bioactive constituents of *Cimicifuga racemosa* using maceration.
2. Evaluation of antioxidant potential of *Cimicifuga racemosa* rhizome extract by FRAP Assay.
3. Investigation of the efficacy of *Cimicifuga racemosa* rhizome extract against cervical cancer HeLa cells.

LITRETURE RIVIEW

CANCER

Cancer is the progression by which normal cells get transformed to malignancy (Hida *et al.,* 2018). This process arises from mutations that damage the genome integrity (Chen et al., 2018). The source of damage can either be endogenous for example; mistakes during DNA replication, free radical attack throughout metabolism, chemical instability of specific DNA bases or exogenous factors such as viruses, ionizing radiation and chemical mutagens. Normally healthy cells have a way of countering such errors but for a number of reasons, these damages go unrectified and end up causing permanent mutations in the genome (Katerji *et al.,* 2021). The mutations commonly affect the genes that are tasked with maintaining the integrity of the genome consequently, they lead to further transformation of normal cells to abnormal ones (Heng *et al.,* 2020).

Since mutations have to be introduced into the human genome for cancerous cells to develop, it is prime to get to know the different pathways that they take on to qualify to be termed malignant (Ganesh *et al.,* 2021). The mutations result into two discrete repercussions; they facilitate the improper expression of genes and on the other hand effect the inactivation of the gene product which is the protein it produces. Usually, mutations trigger oncogenes which function to signal pathways that brace proliferation and deactivate tumor suppressor genes that encode proteins that serve as checkpoints for cell division and death. As a result, uncontrolled proliferation is inevitable in cancerous cells (Ben-David *et al.,* 2020).

Hallmarks of Cancer

Normal cells have many mechanisms that regulate their growth, division and death whereas cancer cells evade all these regulatory systems because they have a transformed physiology (Markopoulos *et al.,* 2017). Below is the cell physiology of malignant cells that enables their existence;

1. Genetic and epigenetic variations

There are six categories of genetic and epigenetic variations that translate to cancer;

1.1 Self-sufficiency in growth signals

Typical cells rely on growth signals that are of highly regulated cell cycle to maintain proliferation and homeostasis (Ginzberg *et al.,* 2018). For instance; normal cells have receptors on their surfaces which depend on external signals such as EGF/EGFR to initiate growth. This cycle is interrupted in cancer. Cancer cells might have excess number of receptors on their surface which when triggered cause unrestrained growth but in other cases cancer cells do away with signaling or depend on their own growth factors to initiate proliferation. The later explains the ability of cancer cells to overcome EGF.

1.2 Evading growth-inhibitory signals

Growth-inhibitory signals regulate proliferation of cells negatively to maintain the fidelity of the genome. These signals include; gene p53 (Blandino *et al.,* 2018) which is tasked to check for errors such as DNA damage during cell cycle and to arrest the cells with damaged DNA at G0 phase till the DNA is repaired, gene RB1 that works by blocking cell division from going on from G1 to S phase at the identification of damaged DNA by regulating the activity of cyclin and cyclin dependent kinase complexes and gene TGF-beta which halts the cell cycle at G1 phase (Sen *et al.,* 2018). Cancer cells to do away with such growth inhibition signals because they lack response to such growth suppressors due to the mutations they have accumulated. As a result, they are able to resist autophagy and apoptosis that are key in controlling cell proliferation and death.

Apoptosis permits the elimination of cells that are undergoing extreme division to maintain cell number and get rid of diseased cells. Autophagy on the other hand, is a cellular recovering system that take away abnormal proteins as well as cytoplasmic materials and encourages regeneration especially during stress conditions (D'Arcy *et al.,* 2019). For this case, cancer cells evade apoptotic signaling and thus avoid death but they encourage

autophagy to facilitate growth and more so be able to outsmart nutrient-limiting environments.

1.3 Avoiding programmed cell death

When typical cells are at the end of their life cycle or a DNA damage is identified at the course of the cycle, it is marked to go through apoptosis (Liu *et al.,* 2020). On the contrary, cancerous cells have intrinsic systems that permit them to prevent cell death through apoptosis. The intrinsic mechanisms are as a result of mutations that stop the detection of damage or avert apoptotic signaling inside the cell leading to uncontrollable multiplication of cancerous genes (Chota *et al.,* 2021).

1.4 Permitting Limitless Replication

Usually, ordinary cells are set to undergo specific number of divisions after which, they cease dividing and get marked for apoptosis (Zhang *et al.,* 2019). Conversely, tumor cells are able to get infinite replication potential by producing hiked levels of telomerase enzyme which maintains the length of telomere by adding more telomeres during DNA replication thus preventing senescence and apoptosis. For this reason, cancerous cells are able to divide infinitely without entering senescence (Mangosh *et al.,* 2021).

1.5 Sustained angiogenesis

Angiogenesis is the development of new blood vessels which is essential during embryogenesis or healing of wound (Noishiki *et al.,* 2019). Since tumor cells need sustained surplus of nutrients and oxygen to keep up with infinite proliferation, they produce chemical signals such as Vascular endothelial growth factor (VEGF) and Basic Fibroblast Growth Factor (bFGF) which stimulate angiogenesis around the tumor cells.

1.6 Initiation of invasion and metastasis

Invasion and metastasis are central marks of malignancy that result to death. They are marked by disintegration of cadherin protein which holds cell to cell and degradation of cell extracellular matrix which is made up of matrix metalloproteases. These events make

it possible for tumor to break off from cancerous sites and move to infect neighboring tissues or organs to start developing new tumors (Das *et al.,* 2019).

2. Microenvironment factors

Tumor cells gain means to disrupt the normal cells and processes that are within their microenvironment with an intention to maintain their growth and metastasis (Baghban *et al.,* 2020). The following are the four microenvironment factors that encourage progression of cancer;

2.1 Evading immune destruction

Most of the small tumors can be identified and eliminated by the immune system. However, the larger ones have a means of avoiding immune detection and likewise immune destruction. This is because once the system identifies tumor antigens, it secrets antibodies to counter them but the replication of cancerous cells is rapid enough such that they outsmart the immune mechanism by mutating to evade growing with the antigens which the body had initially detected and produced their antibodies (Leong *et al.,* 2018). Correspondingly, they go unnoticed by the immune fighting mechanisms. Furthermore, following the rapid growth and division, tumor cells use up oxygen supply and nutrients very fast, increasing metabolism rate and making their microenvironment acidic, thus they attract immune cells that promotes healing rather than the ones that fight tumors.

2.2 Deregulation of cellular metabolism

Tumor cells undergo aerobic glycolysis irrespective of the presence of oxygen. In as much as aerobic glycolysis results in less energy, cancer cells convert glucose to lactate because it leads to overexpression of GLUT1 which allows the cancerous cells to uptake more glucose from the microenvironment than normal cells (Abbaszadeh *et al.,* 2020). Furthermore, tumor cells trigger the biosynthesis of amino acids precursors such as nucleic acids to help in the formation of daughter cells that aid in faster growth and division of cancer cells.

Fast proliferation of tumor cells creates hypoxia which is a state of reduced oxygen concentration (Butturini *et al.,* 2019). This imposes stress to cancerous cells which further cause DNA damage and thus more malignancy.

2.3 Genome instability and mutation

Cancerous cells evolve continuously as their genes keep mutating to adjust to the new surroundings (Fares *et al.,* 2020). The p53 and RB1 are the genes that constantly mutate in tumor cells following the rapid proliferation. These multiple mutations facilitate the cancer cells to evade death signals thus lead to development of high tumors.

2.4 Tumor facilitating inflammation

The microenvironment of cancer cells is populated with immune cells which give out inflammatory signals as cytokines (Dehne *et al.,* 2017). The released cytokines trigger the immune cells to give rise to proteins that encourages angiogenesis which directly supports the growth of tumor (Albini *et al.,* 2018). Additionally, the numerous cytokines in cancerous cells microenvironment make the nearby normal cells to transform into pro-neoplastic mutations that resist cell death (Anuja *et al.,* 2017).

Tumor cells also overproduce cyclooxygenase 2, COX2 which serves as an inflammation mediator thus they protect themselves from cytokines released by immune cells (Chakraborty *et al.,* 2020).

Cervical Cancer

Cervical cancer being the world's fourth most occurring cancer in women has drawn researchers to find out means to mitigate its effects. It has caused alarming deaths over the years all through the globe but its effect is most common in developing countries (Berman *et al.,* 2017). In Kenya, there is an increased frequency of cervical cancer, especially in adults. This may be accredited to a rise in life expectancy collectively with the embracing of unhealthy lifestyles.

Cause of Cervical Cancer

Cervical cancer in many cases is as a result of HPV infection (Cohen *et al.,* 2019). Other risk aspects that contribute greatly to its development include; type of HPV infection, several sexual partners, frequent births, co-occurrence of additional infectious agents, typical smoking and deficiencies of vitamin. Usually, the body's immunity fights off and eliminates HPV infection over a given period of time but this is not the case if an individual is infected with the HR HPV strains (Neunez *et al.,* 2021). Smoking on the other hand lowers the body's immunity by reducing the immune system cells that are tasked to fight infections (Islam *et al.,* 2021). Moreover, smoking releases polycyclic aromatic hydrocarbons (PAH) which are known to be carcinogenic. The PAHs irritates the cervical tissues and in the long run contributes to immunosuppression.

In the opinion of the World Health Organization (WHO), all people who are sexually active will be infected once or recurrently with HPV since it is a sexually transmitted infection (STI). HPV transmission occurs through skin-to-skin contact. This infection is more rampant in the genital areas. Over the years, there has been increased evidence that links HPV to being a cause of cancer in the head, anal, vagina, neck and vulvar (De Martel *et al,* 2017). Bearing in mind that HPV infects all people who are sexually active, only females are in the receiving end when it comes to cancer because male cancers that arise from this infection are uncommon.

Irrespective of the gender that HPV infects, it normally occurs with no symptoms. But in other incidents, it leads to growths on genital areas which cause constant irritation as well as discomfort. Moreover, there are specific lesions that are symptomless. When the latter are left untreated, they advance and progress over time into cancer (Serrano *et al.,* 2018).

When it comes to low-risk types of HPVs such as HPV- 6 and 11, they don't directly lead to cervical cancer but they facilitate the development of condyloma which leads to psychological morbidity that creates stress in the genital tract. Over the years, many patients have been reported to suffer from HPV infections repeatedly. For the aggressive forms of HPV to repeatedly infect an individual, there must be an underlying immune reason. Though it is not yet clear what conditions contribute to this report. However,

probable contributing factors to reinfection has been associated to; numerous sexual partners, inadequate immune function, frequent infection by other STI's and the use of oral birth control devices for more prolonged period of time.

In as much as HPV effects can be treated medically either surgically to remove the warts or by use of various medications to make the lesions go away; it cannot be cured. So, it is important to evade skin to skin contact. Furthermore, there is an HPV vaccine that serves to protect people from the HR HPV (Burger *et al.,* 2021). The vaccine is eligible for any individual from 9 years old all through to 45 years. But depending on the particular age you choose to take the vaccine, the number of doses vary, for example; two doses are given to people who decide to take the vaccine at 9 to 14 years and three shots are taken by those who initiate their vaccination procedure at 15 all through to 45 years as well as to the individuals who have compromised immunity.

Onset of Cervical Cancer

Cervical cancer affects the surface of the cervix which is the neck of the vagina. It is brought about by the transformation of normal cells on the cervix area into precancerous cells (Berman *et al.,* 2018). Usually, not all precancer cells translate to cancer but leaving them untreated over a long period of time will qualify them to cause cancer. This suggests that it is prime to identify and treat such cells at their onset before they become worse. Nonetheless, it is difficult to point out these abnormal cells as they are tiny and can only be detected through cervical cancer screening. Typically, this calls for women to go for regular examination as it is a crucial step for preventing the progression of oncogenic cells to cervical malignancy.

When it comes to cervical cancer screening, two methods are commonly employed; the Pap test and HPV test (Chrysostomou *et al.,* 2018). Pap test, which is notably known as Pap smear checks for cell changes on the cervical area that might advance overtime and effect the onset of cancer. Similarly, HPV test is aimed at identifying the presence of HPV strains that cause cervical cells to transform to malignant cells.

HPV Structure

HPV is a small, non-enveloped, icosahedral DNA virus that is of 52-55nm in diameter. This virus is made up of a single DNA molecule of 8000 base-pairs (bp) which is attached to cellular histones. It has a protein capsid that is made up of 72 pentameric capsomers (Zehra *et al*., 2022). That capsid encompasses two structural proteins; the late 1(L1) and late 2 (L2) which are virally encoded. The L1 makes up most of the capsid and can solely amass into an empty virus like particles. On the other hand, L2 protein is a minor part of the capsid which serves in the assembling of papillomavirus and likewise in the infection process.

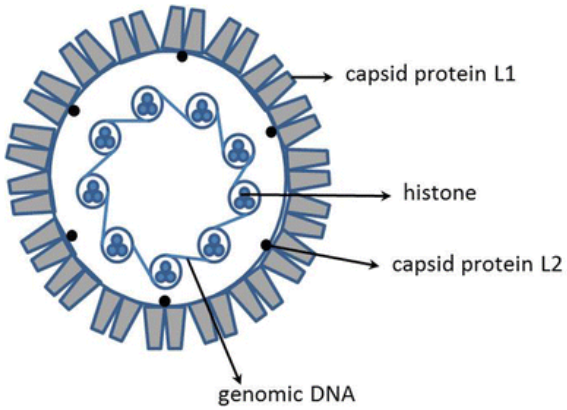

Figure 1: *Structure of HPV*

Pathogenesis of HR HPV infection

HPVs infects the human body by penetrating through any opening on the skin, be it a cut or scrape. But majorly, it enters the body through sexual intercourse. These viruses are limited to infecting basal cells of epithelium tissues at several sites of genital tract such as; vagina, cervix and vulva. This is because HPVs cannot attach themselves to live cells, they depend on small abrasions that exposes segments of the basal membranes. By utilizing the L1 protein HPV gains attachment to the heparin sulphate proteoglycans and in other instances, it comes in contact with laminin. Conformational variations in viral capsid as a result of L2 furin cleavage permits the virus to bind to the keratinocyte receptor, which is key for viral internalization (Guion *et al.,* 2019). HPV viral proteins E1 and E2 are in control for conserving a small number of genome copies because viral genomes are transported to the host nucleus and get established as episomes. Early

viral genes; E1, E2, E6, and E7 are expressed and thus the viral genome gets replicated just as the normal cell's DNA. As soon as the cell division is done, the infected daughter cell starts the progression of keratinocyte differentiation that facilitate a successful pattern of viral gene expression for a productive infection. The differentiated infected cells, produce E4 protein which encourages intensification of viral genome replication. Proteins of late genes L1 and L2 are required for the formation of new virions, which keep infecting the cornified layer of the epithelium.

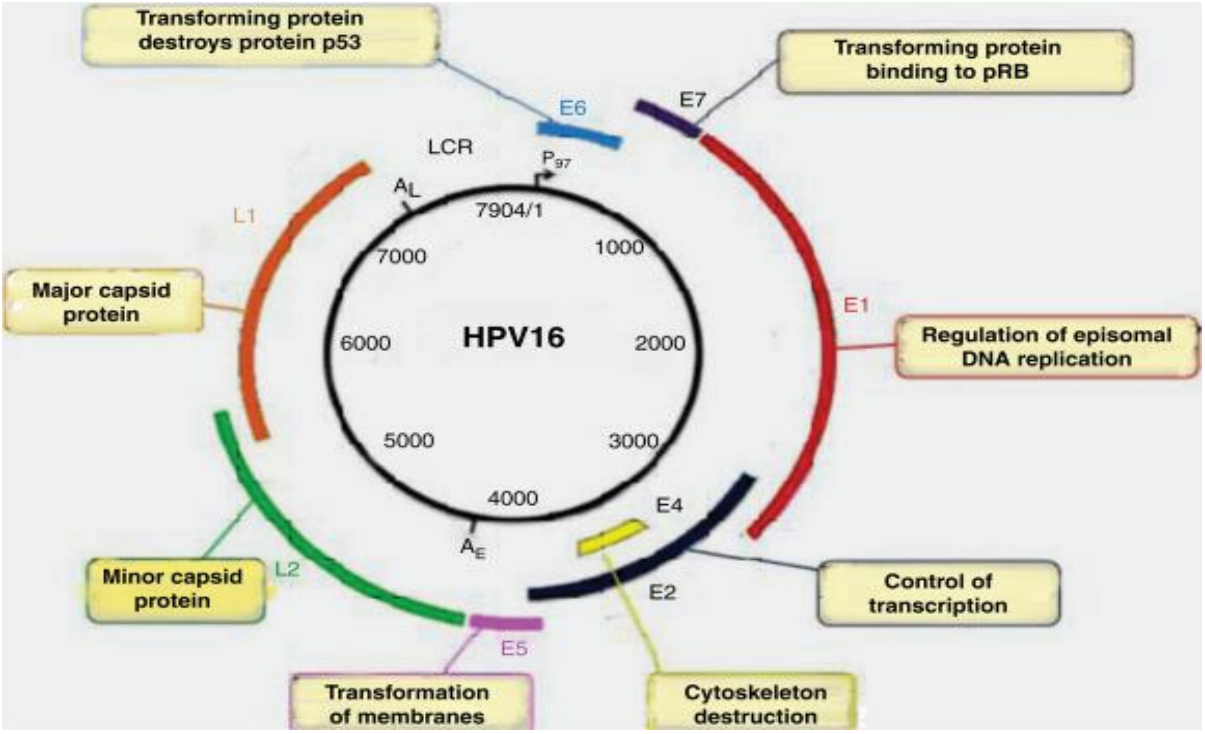

***Figure 2**: Structure of HPV 16*

During the successive HPV infection, the E6 and E7 gene proteins serve to disrupt the normal cell growth-regulatory mechanisms by modifying the cellular environment so that it can support viral replication in cells that have undergone terminal differentiation and have exited the cell cycle (Zheng *et al.,* 2022). Normally the cell cycle is controlled to greater extent by two tumor suppressor proteins; p53 and the retinoblastoma gene product. The E7 turns out to be the primary transforming protein because it contests for retinoblastoma protein binding and in the process, it releases the transcription factor E2F to transactivate its targets and correspondingly push the cell cycle forward. Similarly, the E6 in connection with host E6-associated protein that bears ubiquitin ligase activity,

functions to ubiquitinate p53, making it to undergo proteasomal degradation (Drews *et al.,* 2019). This condition leads to oncogenesis and the development of mere lesions to carcinomas which end up as either squamous cell or adenocarcinomas cervical cancer.

Types of cervical cancer

The cervix has an interior cavity that is known as the cervical canal which has two sections; the endocervix and the ectocervix. Endocervix is cervix part which is in close proximity to the uterus and hence cannot be visualized by naked eye, it is lined by columnar epithelial cells that secretes mucus. The Ectocervix on the contrary is linked to the vagina and is packed with mature squamous epithelial cells. For this purpose, the intersection where the squamous epithelium of the ectocervix and the columnar epithelium of the endocervix joins, it is termed as squamocolumnar junction and the exact location where these types of cells meet, there is a transformation zone (Grover *et al.,*2017).

When one has mutated cells, the transformation zone provides an avenue where the sub-columnar reserve cells multiply and transform into immature squamous epithelium cells through a progression known as metaplasia (Ojha *et al.,* 2022). In the normal cell cycle, the mature cells get stuck in the G1 phase for them to grow effectively and take care of the regular cellular activities such as the synthesis of proteins which are used to make hormones and production of energy. Eventually, when the body system requires new cells, the mature cells exit the G1 phase and follow through all the cell cycle stages before finally dividing to form two new undistinguishable daughter cells. Equally important, there are instances when cells are forced out of the G1 for them to go through reproduction process in a rapid manner than how the body require new cells. This untamed growth and multiplication are known as dysplasia and it is the key process for multiplying precancerous cells that end up causing cervical cancer (Reynolds *et al.,* 2022).

With this in view, when dysplasia occurs in the epithelium layer of the cervix, it is called cervical intraepithelial neoplasia or alternatively squamous epithelial lesion (Berman *et al.,* 2017). This is specifically as a result of the disease starting from the basal layer of the

transformation zone, the immature squamous epithelium. In most instances, this type of cervical cancer is as a result of HPV infection which inserts itself on the undeveloped squamous cells in the transformation zone and then fits in its DNA into the host DNA. The Infection then uses the host DNA to produce large amounts of its proteins; the E6 and E7 which are in charge of pushing the mature squamous cells in the G1 phase to go into cell replication process regardless of the body not requiring new cells. These proteins are successful in upsetting the normal systems in the cervical region because they block the action of the tumor suppressor genes, such as the p53. For this reason, there is uncontrolled proliferation of the cervical epithelial cells that have the mechanism to evade apoptosis.

The higher the level of infection of the transformed cells over the epithelium cell, the more the dysplasia evolve into malignancy (Deng *et al.,* 2018). To this end, it is evident that HPV tends to attack the squamous cells first and as a result, many cervical cancer cases are squamous cell carcinomas. In like manner, there is a second most occurring type of cervical cancer, the adenocarcinoma which is also linked to HPV infection. In particular, this later type arises from the epithelial gland cells of the cervix which are the secretory organs for producing mucus (Ciu *et al.,* 2018).

Diagnosis of Cervical Cancer

There are a number of tests that are available for diagnosis of cervical cancer. Either of the following can be used on a patient depending on the symptoms they are portraying, earlier feedback from other medical examinations and patient's age and general health issues.

Bimanual pelvic examination

This procedure focuses on finding any uncommon changes in the cervix, uterus, vagina, ovaries as well as the neighboring organs. It begins with external examination of any rare occurrence on the vulva then by use of a speculum to maintain the walls of the vagina open, the doctor checks the internal structure of the vagina to look through to the cervix. Usually, a Pap test accompanies this procedure to help identify abnormal cells. If the

nearby organs are difficult to visualize, the doctor inserts two fingers of one hand onto the vagina and uses the other hand to softly press on the lower abdomen to feel the texture the uterus and ovaries.

Pap test

This procedure involves the scraping to collect samples for cytological testing from both the inside and outside the cervix. It aims at preparing the samples for visualization to identify the presence of abnormal cells in cervix. If the tests come out positive, then a patient is prepared for colposcopy.

HPV typing test

It is molecular analysis carried out on samples collected from cervix just as in the case of Pap test. It majorly facilitates the identification of the presence of aggressive HPVs such as HPV-16 and HPV-18 since they are the common strains in cervical cancer patients. When the results come out as positive, it implies that the patient has high-risk HPV. However, this test alone does not prove that a patient has cervical cancer.

Colposcopy

Colposcopy is an examination of the cervix to identify abnormal tissues. It commonly helps to direct a cervix biopsy and it makes use of a colposcope to enlarge cervix and vagina cells by giving them a high resolution. Following this, the doctor gets an amplified view of the tissues in the vagina as well as the cervix.

Biopsy

This is a definite diagnosis for cervical cancer. It involves the removal of a small tissue from the cervix for analysis under a microscope by a pathologist. The pathologist, examines the samples under the microscope to check for abnormal cells and tissues. There are several biopsy types that a doctor can carry out for example; Endocervical curettage which utilizes samples scraped off from the inside of a cervix, loop electrosurgical excision procedure which examines samples that have been removed from the cervix using an electric current loop and conization that examines a cone-shaped tissue removed from the cervix. Once a patient turns positive for cervical cancer, the doctor recommends them to a gynecologist oncologist.

Cystoscopy

This is a further examination that utilizes a cystoscope to visualize the bladder and urethra after a patient has been diagnosed with cervical cancer. Its intention is to determine if the malignancy has spread to the bladder.

Sigmoidoscopy

This procedure employs the use of a sigmoidoscope to check the rectum and colon. It is yet another further examination to analyze the extent to which cancerous cells have spread. This method allows proper staging of cervical cancer which helps in determining the type of treatment which is effective to the patient.

Classification of Cervical Abnormalities

For classifying the abnormalities of cervical cancer, Cervical Intraepithelial Neoplasia [CIN] procedure is applied to determine how much of the epithelium has been affected by the disease. The CIN identifies the dysplasia growth and progression of abnormal cells. During this examination; CIN I stand for minor dysplasia that has affected only the lower one-third of the epithelium in terms of thickness, CIN II corresponds to moderate dysplasia that has affected two-thirds, CIN III links the results to extreme dysplasia, where almost all of the epithelium is affected also referred to as pre-invasive carcinoma and finally carcinoma in situ which affects the entire thickness of the epithelium (Shiraz & Majmudar, 2017).

At the later stage of disease progression, the in-situ carcinoma transforms into invasive cervical cancer which is the lesions that have the mechanism to break through the epithelium basement membrane tissues and into the cervical stroma and begin spreading to the nearby tissues such as the epithelium layers of the uterus and the vagina (Balasubramaniam *et al.,* 2019). Accordingly, it can pass through the pelvic wall and infect other vital organs such as the bladder and rectum. Similarly, it uses the lymphatic and circulatory systems to spread to distant organ systems such as the lung and liver.

Signs and Symptoms of Cervical Cancer

Cervical cancer at first stage is characterized by abnormal vaginal bleeding particularly after sexual intercourse. Later on, it can progress to vaginal discomfort, vaginal discharge with off odors and pain when urinating. With the result of cancer

spreading past the pelvic wall, the symptoms intensify to constipation and bloody urine (Otero-García *et al.,* 2019).

Treatment of Cervical Cancer

The treatment possibilities for cervical intraepithelial neoplasia include; cryosurgery where a liquid nitrous oxide is employed to freeze and kill cancerous cells, conization procedure which entails the surgical removal of the transformation zone or all parts of the endocervix.

In conization procedure; several devices can be utilized, for instance cold-knife conization for removal of the cone-shaped tumor from the cervix, laser technique which make use of laser beam to burn out the cancerous cells and electrically heated loop electrosurgical excision procedure which is a times referred to as a large loop excision for the removal of the transformation zone. In other cases, the doctor may do simple hysterectomy procedure which is particularly intended to surgically remove the uterus without touching the tissues that are adjacent to the uterus such as the vagina and the lymph nodes.

Following the extent of the tumor spread over the epithelial layer of the cervix or onto the neighboring tissues, other surgical methods can be incorporated to eliminate the malignant cells for instance; Trachelectomy which focuses on the elimination of the cervix alongside the upper part of the vagina without disturbing the uterus, Radical hysterectomy procedure where the entire uterus plus the surrounding tissues such as the parametrium, cervix, some upper portion of the vagina and the entire lymph nodes from the pelvic region are cut off and Pelvic exenteration process which is an advanced surgery after the radical hysterectomy when the cancer has gained metastasis, this procedure aims at removing the bladder, vagina, rectum and some part of the colon area.

In the instances where the tumor has advanced greatly or reoccurred, patients are booked in to undergo radiation and chemotherapy which are techniques put in place for killing cancerous cells in the body system although they end up injuring the normal cells in the long run. When a patient is diagnosed of stage IV cancer, it is beyond medical intervention, it is incurable. The only thing that can be done at the final stage

is to manage the patient. For this reason, the earliest cancer stages are able to be mitigated medically.

At any rate, the cancer treatment providers give alternatives that form combination of treatments to cervical cancer patients to try and manage the effect and the spread of tumor (Cibula *et al.,* 2018). These treatments apart from surgery, are targeted therapy, immunotherapy, clinical trials and alternative treatments.

Targeted therapy

Targeted therapy is the utilization of a particular drug that has destructive effect precisely on given cancer cells. Without a doubt, it grants the cancer patients' healthy cells a chance to continue thriving as it avoids damaging them. This form of treatment aims at destroying some proteins that regulate how cancer cells proliferate and spread. As researchers study more about the complexities that accompanies cancer as a disease, they are able to develop improved targeted treatments that specifically eradicate such proteins (Senapati *et al.,* 2018).

Immunotherapy

Immunotherapy is the use of certain medicine to stimulate the patient's immune system for it to be able to quickly identify tumor cells and launch a mechanism to eliminate them from the system. Notwithstanding that cancer cells possess the ability to send off signals that block the immune system from attacking them, immunotherapy aim at such signals so that the cancer cells lose the capability to trapping the body's immune system into assuming them as healthy cells (Dine *et al.,* 2017).

Clinical Trials

Clinical trials as an additional treatment opportunity are controlled research studies for testing novel cancer treatments (Piantadosi *et al.,* 2017). Any cancer patient can participate in it after consulting their oncologist.

Alternative treatment

Cancer patients over the years have adapted a number of alternatives to mitigate the symptoms of the disease they are suffering from. These include; the use of specific diet and herbs to supplement their cancer medication. The alternative treatment may or may not give positive results.

Altered Signaling Pathways in Cervical Cancer

Understanding the signaling pathways in CC helps in reevaluating the available therapies in order to come up with effective ones to ensure general cancer survival. The theory of cancer stem cells (CSCs) is the ongoing concept in developing novel approaches to this disease (Crous *et al.,* 2018). CSCs are relatively smaller portions of tumors which comes from progenitor or cancer cells and are able to undergo fast symmetric and asymmetric division to self-renew and result in differentiated progeny. Therefore, they facilitate initiation of a variety of new tumors which are resistant to conventional therapies and lead to relapses (Liu *et al.,* 2020).

CSCs have the ability to spread cancer to other organs throughout the body, as they have the mechanism that allow them to break away from the large tumor and enter the blood stream to circulate around the body and finally invade distant tissues (Liu *et al.,* 2020). Once they get attached to the new tissues, they start processing their genome from scratch. This majorly contributes to the hiked number of global cancer deaths.

As mentioned in this paper, CSCs are resistant to conventional cancer therapies, however, current therapies tend to effectively kill the fast-proliferating cells on tumors (Rossi *et al.,* 2020). On the other side, the slow replicating CSCs have been spared by such therapies and such has granted the regrowth of the tumor. Hence, there is need to develop new therapies that kill the CSCs. According to research, specific CSCs therapies may not serve as the cancer cure because the bulk of the tumor cells may transform and gain the mechanism to go back to being cancer stem cells which drive to tumor regrowth. For this reason, specific CSCs therapies should be combined with therapies which are intended for bulk tumors to target all the cancerous cells and destroy them once before

they get a chance to convert back into CSCs. This strategy will go a long way in increasing the number of cases that can be cured for good. To effectively formulate efficient cancer therapies, its prime to understand the signaling pathways which are used by CSCs (Phi *et al.,* 2018). These pathways are similar to the ones in normal stem cells. They include; Wnt, Notch, Hedgehog (Hh) and apoptosis

NOTCH Signaling Pathway

As the name suggests, Notch pathway resulted from a receptor known as notch that is found on the surface of the cell membrane. The Notch receptor is a single membrane spanning multi subunit- containing protein which is found on the cell membrane. Notch signaling is a highly regulated pathway in the organism since it is key in vital development and maintenance of cellular homeostasis. It is involved in cell-to-cell communication. It directly plays a role in cellular replication, differentiation and apoptosis of stem cells. Given that it exists in both cancerous and normal cells it plays dual functions; in ordinary cells it leads to cell death and tumor suppression while in cancer cells it quickens the growth of tissues which results to more tumors (Zhou *et al.,* 2022).

The onset of cancer is as a result of cellular process that imitates the normal cell proliferation but is partially initiated by mutations in the DNA. The mutations in numerous molecules that are in control for cell determination are termed to be oncogenic, but less information is available to explain the participation of typical cell fate-determining systems in oncogenic progression (Marei *et al.,* 2021). The Notch pathway outlines preserved mechanism which is an overall cell interaction machinery that oversees the essential characteristics of cell determination through both vertebrate and invertebrate growth. In cervical cancer, human Notch pathway portrays a cellular setting where cell fate alterations happen and characterization of neoplastic conditions. Based on research, the existence of Notch is proved by the existence of numerous cells that go through cell fate variations. For this reason, Notch pathway can be utilized to evaluate the presence of cell fate irregularities in cervical cancer and more so in other epithelial neoplasia (Fowler *et al.,* 2022).

Cancer of the cervix being the common occurring malignancy and the fourth disease to affect women globally has been linked to HPV infection. According to research, HPV infection takes a long time before it forms enough mutations that leads to malignant tumors. For this purpose, further studies to try and give evidence on how supplementary cellular events from normal cells aid in maintaining cervical carcinogenesis all through the long period are still ongoing.

In human cervical cancer, there is the presence of Notch1 pathway which portrays dysregulated signaling. It is made up of constituents of proteins which make up the trans membrane receptors that integrates signals to support cell fate determination, propagation and cell death. The Notch receptor interrelates with ligands from jagged protein family and Delta-like protein family. Since the Notch pathway involves intercellular signal interactions, it involves two cells, one cell sends the signal while the other receive. This interrelation results in two consecutive proteolytic cleavages which leads to the release of the cytoplasmic section of Notch (Notch-IC). The resulting cleaved intracellular nucleus of Notch C-terminal portion translocate to the other cell nucleus and assemble CSL (CBF1, Suppressor of Hairless, Lag1) proteins which transcriptionally control target genes such as Hes1, Hairy Enhancer of Split (Zhou *et al.,* 2022).

Based on collective observations that have suggested that Notch signaling principally overlap with the development of superior precursor abscesses that transform to invasive cervical tumors, the following features have been proved to take place; build-up of intracellular portions of Notch1 that are characterized by a higher expression of Jagged 1 protein, downregulation of Manic Fringe which is a negative controller of jagged 1-Notch signaling, and hyperactivation of Hes1.

Additional Notch signaling pathways have also been proved both In vitro and in vivo to show collaboration action with HR HPV oncogenes; E6 and E7 that leads to progressive transformation to form high-grade tumors (Zheng *et al.,* 2022). Nonetheless, boosted in vitro and in vivo alteration that triggered Notch1 alleles demonstrated an oncogenic form of E6 (83 aa) that builds up during the development of CC.

Moreover, PI3K-AKT and Myc have been identified to be the probable mediators of Notch pathway in the framework of transformation functions. Alternatively, stimulated Notch signaling has been identified to knockdown cell development in human CC cell lines. Extensive studies have revealed the dose-dependent consequence that exists in Notch signaling thereby, suggesting that Notch pathway ensures collaboration with oncogenes E6 and E7 of HPV in primary keratinocytes in xenografts (Zheng *et al.,* 2022). This suggests that inhibition of Notch pathway will correspond to inhibition of growth of cervical cancer.

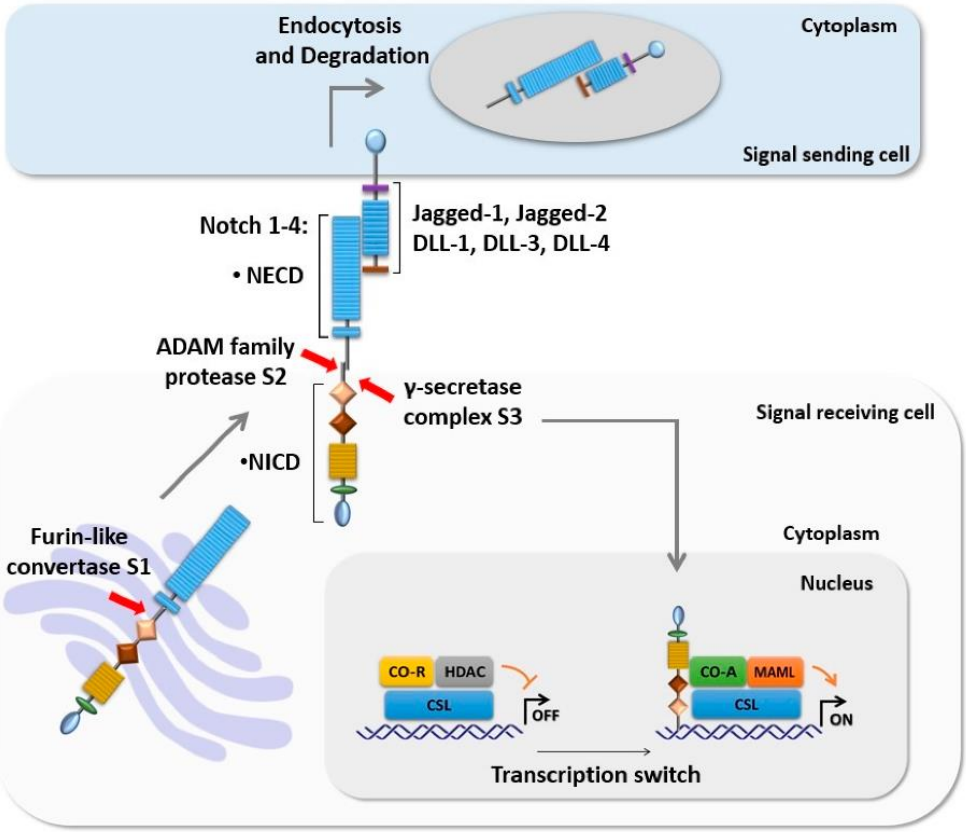

Figure 3: *Notch signaling pathway: Initiated when the notch receptor gets stimulated following the binding of Delta or Jagged protein ligands via cell-to-cell interaction.*

Alteration of Notch Signaling

Notch signaling is tasked to control specification of cells and homeostasis of compartments of stem cell during embryogenesis but it is thwarted by Numb which is a cell fate determinant. Mutually Numb and Notch are involved in growth of tumors (Kim *et al.,* 2017). When the notch pathway is altered in large carcinomas which are often termed as no-small-cell lung carcinomas (NSCLCs) it results to death of cancer patients. When such NSCLCs are examined, they prove to have lost the Numb expression which in turn cause hiked notch activity. Equally, the studied NSCLCs have shown to possess NOTCH-1 gain of function mutations.

Activation of Notch has been associated to poor clinical products in NSCLC patients who lack mutations in the TP53. Alternatively, primary epithelial cell cultures that are obtained from NSCLC that stops the triggering of the Notch pathway, have selectively been killed by Notch inhibitors because Notch give rise to inhibitors. For this reason, it is evident that the proliferation of tumors is reliant on Notch signaling. In the same way, the development of therapies that targets the interference of Notch signaling activation will give promising therapeutic results in treating NSCLC.

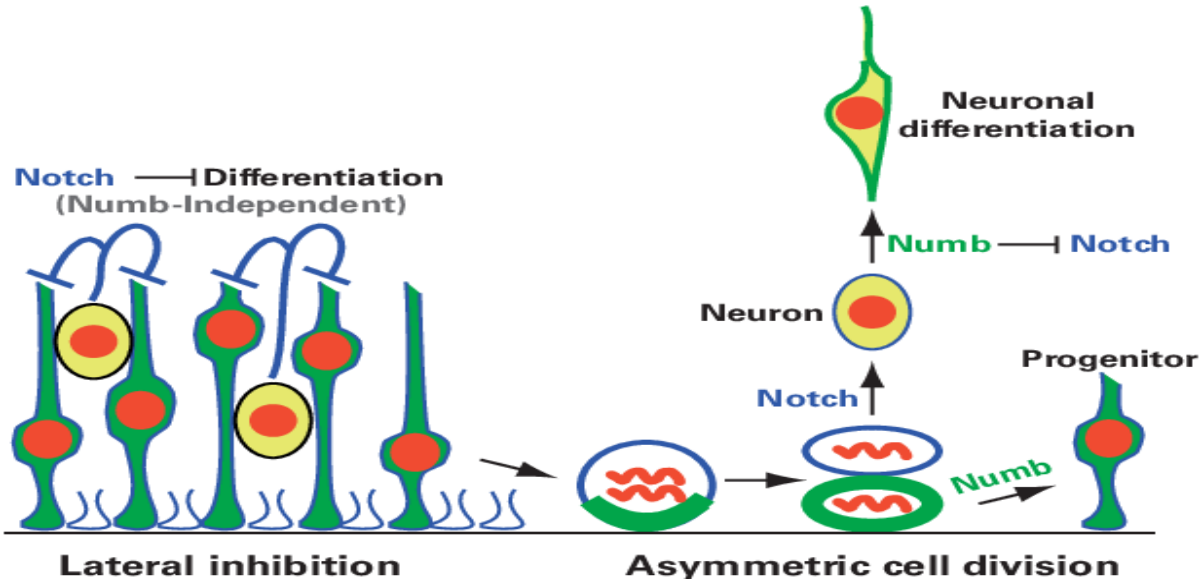

Figure 4: *The enigma of the Numb-Notch relationship during mammalian embryogenesis*

HADGEHOG SIGNALING IN CERVICAL CANCER

Given that cervical cancer is among the leading diseases globally, its incidences are slowly going down in developed countries due to availability of efficient diagnostic procedures that identifies CC at early stages. But this is not the case in developing countries since there is limited diagnostic programs to efficiently identify CC at its early stages. The primary agent for initiation of CC is HPV infection, which is characterized as a small non-enveloped virus of papillomaviruses family. In as much a significant percentage of CC patients have tested positive for HPV, a mere infection of HPV is not enough to cause cancer. Further cellular changes that results in transformation of normal cells to oncogenes must take place to initiate cancer (Wang *et al.,* 2019). In order to recognize the specific cellular variations that take place for CC to develop, we analyzed the effect of the Hedgehog (Hh) signaling pathway in CC.

The Hh pathway is among the central developmental signaling pathways that are key in conserving congenital integrity (Shafi *et al.,* 2020). In cases where this pathway is affected by mutation it leads to congenital malformations. This pathway is stimulated when the Hh ligand binds to its receptor which is identified as patched (Ptch). On binding, it leads to the liberation of second receptor, the Smoothened (Smo), which is normally held in repression. The released receptor proceeds to the membrane and activates a sequence of reactions which are effective in translocation of transcription activators. The translocated activators are encoded by glioma and have oncogenic effect, they include; Gli1, Gli2 and Gli3. The activators are moved into the nucleus to initiate succeeding transcription of the genes of interest. This pattern has been identified to being utilized in cancerous cells.

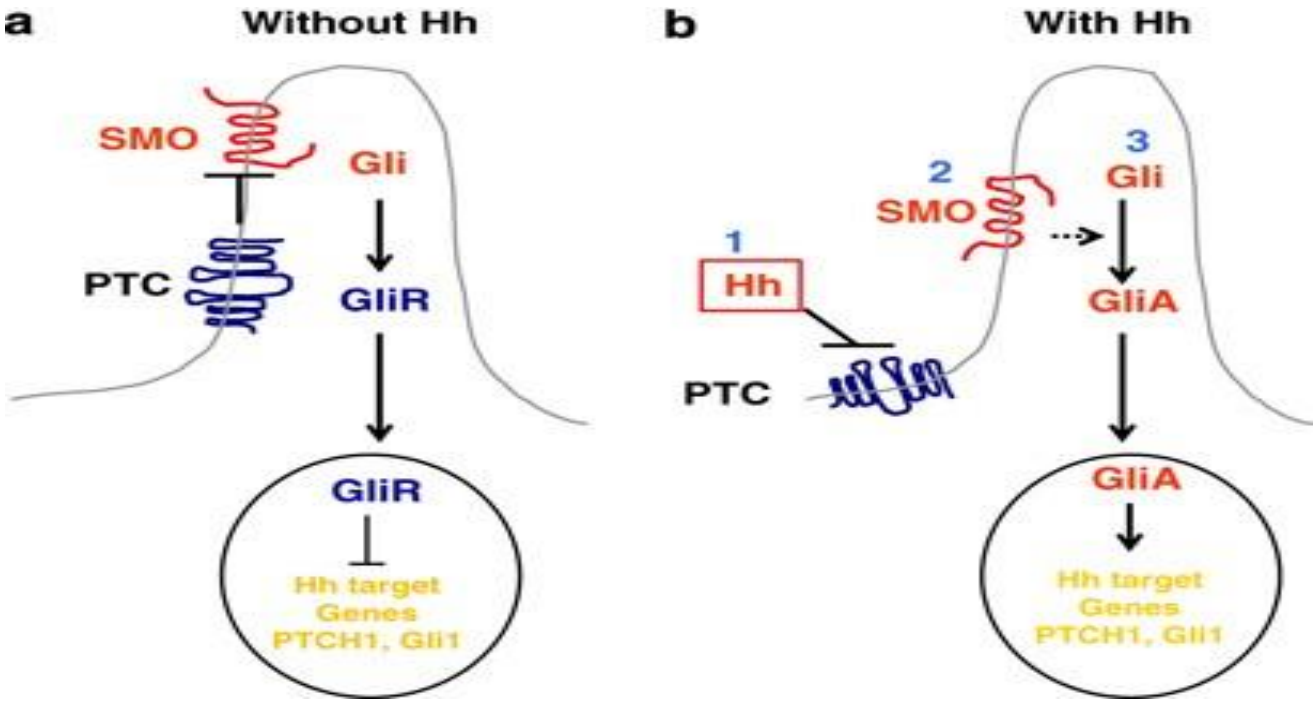

Figure 5: *A schematic of the Hh signaling pathway in the (a) absence and (b) presence of Hh ligand.*

ALTERATION OF HEDGEHOG SIGNALING

Hh signaling pathway plays a role in carcinogenesis that results to development of cancer. It manages to create mutations in genes through two kinds of mechanisms (Xu *et al.,* 2019). The first mechanism is through mutation of constituents that make up the pathway, for instance the alteration of Ptch1 and Smo genes in basal cell carcinoma. This scenario can also involve transformation of Ptch1 alongside the Suppressor of Fused (SuFu) gene which is located in medulloblastoma and rhabdomyosarcoma. The second mechanism by which Hh pathway initiate alteration is autocrine linked, it makes use of Hh ligand just as portrayed by small-cell lung carcinomas, Gastrointestinal tract cancers and breast cancers.

According to recent research, the Hh pathway constituents are progressively overexpressed when the normal epithelium transforms to form squamous cell carcinomas. This clearly signify how Hh pathway molecules contributes to the advancement of normal cells to cancer.

In this paper, we have analyzed the contribution of Hedgehog pathway in cell division, apoptosis and movement using a group of CC cells that are either prompted by HPV altering genes or not and the primary keratinocytes which are non-tumorigenic. By studying the recombinant Shh ligand and pathway inhibitors it has been noted that meddling with the pathway decreases survival, proliferation and migration of CC cell lines. Additionally, it been realized that Shh ligand encourages proliferation and migration of some specific CC cell types.

Initially aberrant Hh signaling and cancer were linked together in the detection of the rare condition Gorlin Syndrome which is essentially as a result of mutation in Ptch124. Patients suffering from Gorlin Syndrome have several basal cell carcinomas (BCCs) developing during their natural life and are also susceptible to extra types of cancers, for instance; medulloblastoma which is a tumor occurring in cerebellar granule neuron progenitor cells, and rhabdomyosarcoma, a muscle tumor (Neves et al., 2018). Furthermore, it has been determined that majority of intermittently occurring BCCs involve hyper-activated Hh signaling because those tumor cells portray the presence of high levels of mRNA of the GLI1 and Ptch1 which are Hh target genes.

WNT/ß-CATENIN SIGNALLING PATHWAY

This pathway is important in varied aspects of cell cycle. For instance; in cell proliferation, fate description, polarity and in movement of cells. Wnt also plays a basic role in maintaining ovarian function, in ovary embryonic development as well as in development of normal follicles (Li *et al.,* 2021). When the Wnt signaling pathway is over activated, it leads to oncogenesis in a number of human organs for example; the liver, cervix and ovary. There are two different pathways via which Wnt signals are transduced; a canonical Wnt/β-catenin pathway and a noncanonical pathway which is independent of β-catenin (Pia *et al.,* 2017). Normally a Wnt ligand binds to a frizzled family receptor which then leads to β-catenin stabilization and its translocation into the nucleus. This transcription factor comes in play in cancer pathways because it transactivates a number of target genes such as MYC and Cyclin D1 that are central in oncogenesis.

Canonical WNT/β-Catenin Signaling Pathway

This pathway serves in regulation of the stability of transcription co-activator β-catenin and as a result it stimulates the expression of a group of target genes, which further controls the proliferation, behavior and life cycle of the cell. When the canonical WNT signals are absent, cytoplasmic β-catenin switch off rapidly following the activity of a multiprotein destruction complex which is made up of AXIN and adenomatous polyposis coli (APC) proteins. The protein complex permits a well-organized β-catenin phosphorylation by the action of casein-kinase 1α (CK1α) as well as glycogen synthase kinase (GSK3β). This phosphorylation leads to consequent ubiquitination and proteasomal breakdown of β-catenin. As a result, there is less levels of free cytoplasmic β-catenin which facilitates the collaboration of T-cell factor/ lymphoid enhancer factor (TCF/LEF) group of transcription factors alongside transcriptional co-repressors Grouchos to suppress exact TCF-responsive target genes (Lu *et al.,* 2020).

At the instances when there are canonical WNT signals, the attachment of WNT to Fzd receptors alongside low-density lipoprotein receptor-related protein (LRP) co-receptors encourages Dishevelled (Dvl)-dependent phosphorylation of LRP. When LRP gets phosphorylated, it takes up AXIN from the destruction complex all the way to the plasma membrane. These events facilitate β-catenin to evade phosphorylation and gain cytosolic stabilization (Brunt *et al.,* 2018). Once the β-catenin escapes phosphorylation, it amasses in the cytoplasm and moves to the nucleus, where it attaches to a transcription activation complex with associates of TCF/LEF family through the displacement of Grouchos and recruitment of other co-activators which includes; BCL9, Pygopus, CBP and Hyrax. The TCF/LEF-β-catenin nuclear complex serves as an enhancer of canonical WNT signaling to trigger the transcription of WNT target genes such as c-Myc, cyclin D1 (CCND1), DKK1, WISP1 and FGF20. The WNT target genes oversee the proliferation, behavior and life cycle of the cell. For this reasons, appropriate regulation of WNT/β-catenin signaling pathway by obstructing β-catenin breakdown to enhance stabilization or any events that aim at degradation of β-catenin is critical for maintaining suitable embryogenesis as well as adult tissue homeostasis.

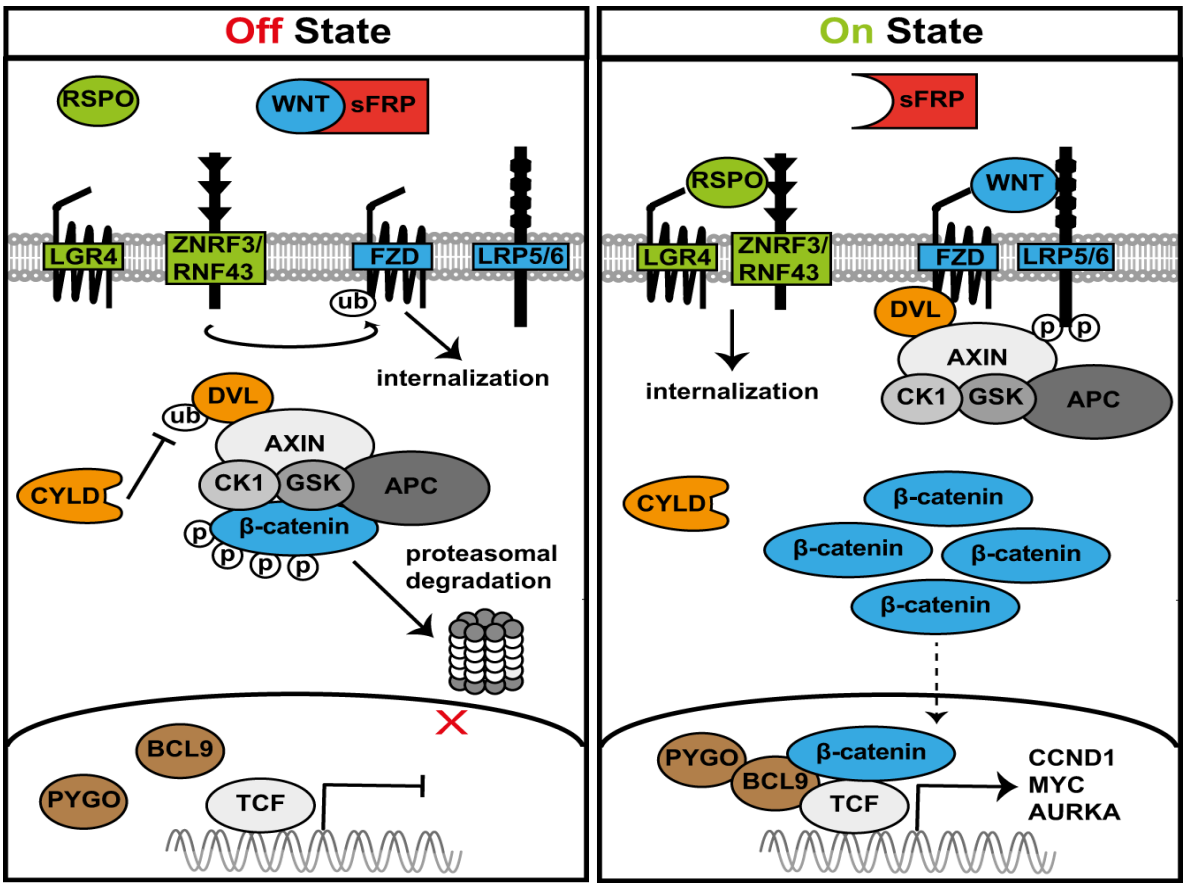

Figure 6: *Aberrant WNT/β-catenin signaling in multiple myeloma.*

Non-Canonical WNT Signaling Pathway

This signaling pathway is not clearly highlighted but it is autonomous of β-catenin-mediated transcriptional initiation triggers. It utilizes other pathways such as planar-cell-polarity (PCP), calcium ion-dependent and JNK pathway (Lojk *et al.,*2021). This WNT pathway works in various methods to control adhesion, polarity, death and migration of cell.

APOPTOSIS

There are two key mechanisms of cell death; necrosis and apoptosis. Cells that get injured by external force go through necrosis, while cells that get triggered either by external or internal stimuli to enter death phase which is normally referred as programmed cell death undertake apoptosis. Apoptosis is steered by various complex proteins which

become stimulated by various stimulations and get organized in sequential signaling modules (D'Arcy *et al.,* 2019).

Apoptosis ensues over two major pathways; the extrinsic and intrinsic. Extrinsic pathway which is also known as cytoplasmic pathway is initiated through the first apoptotic signal (Fas) death receptor which is an associate of the tumor necrosis factor (TNF) (Malhi *et al.,*2020). On the other hand, the intrinsic pathway which is also called mitochondrial pathway gets initiated through the stimuli that permits the release of cytochrome C from the mitochondria. The two pathways after initiation stage, unite to an ultimate mutual pathway which involves the stimulation of a group of proteases called caspases that function in cleaving regulatory alongside structural molecules and concluding in cell death. Since the pathways link up, the difference among them is slight. Alternatively, there have been studies that show that over release of Bcl-2 during intrinsic apoptosis pathway may cause the repression of extrinsic facilitated apoptosis; on the contrary, TNFα may upsurge the production of NFκB and trigger antiapoptotic members of the Bcl-2 family proteins (Wu *et al.,* 2018).

The Receptor-Mediated Extrinsic Pathway

In this pathway, the death receptor is tasked to convey extracellular death signaling molecules to the apoptotic intracellular apparatus to occasion cell death. Death receptors encompass a subgroup of TNF receptor superfamily which is marked by discrete protein motifs, that is death domains (DD) and death effector domains (DED). The specific domains are proficient for monovalent, homotypic interactions. On the external part of the cell there are related ligands from the TNF family CD95, Fas/Apo1 and TNF-related apoptosis-inducing ligand (TRAIL) which interact with one of the main death receptors to entice the DD- containing molecules which include FADD (Fas-associated death domain protein) and/or TNF receptor-associated death domain (TRADD). Recruitment of FADD activates pro-apoptotic pathways while TRADD encourages anti-apoptotic signals. FADD recruits other DD/DED-containing proteins namely; pro-caspase-8 and -10 to endorse the formation of death-inducing complex (DISC) in the cytoplasmic section (Zhou *et al.,* 2017).

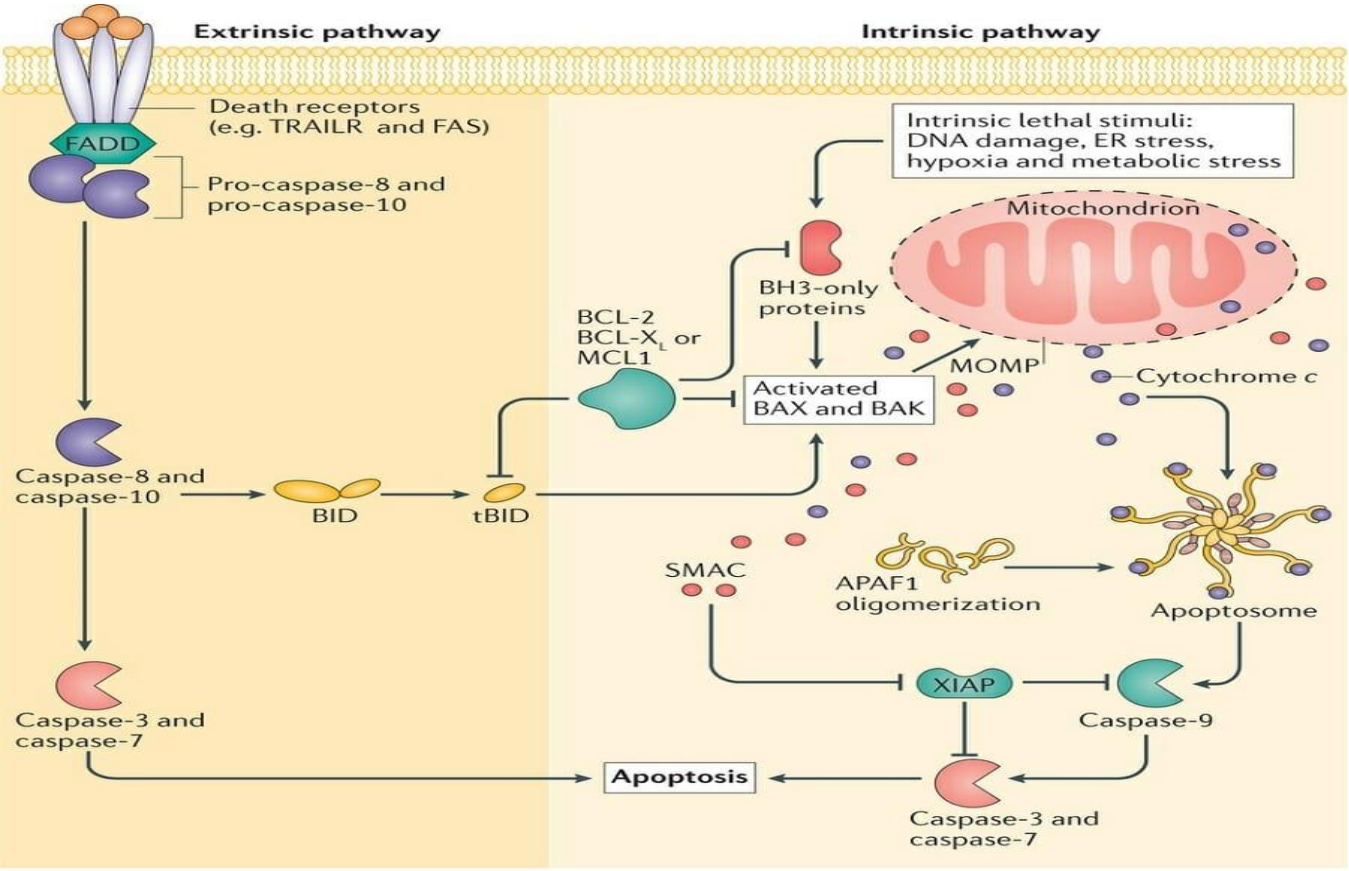

Figure 7: *The extrinsic and extrinsic apoptosis pathways* (Lowe, 2000)*. Both pathways converge at an ultimate common pathway which entails the activation of caspases that degrades regulatory and structural molecules and terminate in cell death.*

On the other hand, TRADD fixes itself to receptor interacting protein-1 (RIP1), TNF receptor-associated factor-2 (TRAF2), TRAF5 and a repressor of apoptosis protein-1 and 2 (c/AP1/2) and forms complex 1. This complex controls pro-survival stimuli which is similar to the one facilitated by NFkB, JNk and p38. Nevertheless, in other situations, RP1 gets deubiquitinated by the enzyme, cylindromatosis (CYLD), which initiate the detachment of RP1 and TRADD from complex 1. For this purpose, RIP1 and TRADD then get fixed to FADD and caspases-8 and -10 to form another complex, complex 2. As soon as caspases-8 and -10 are stimulated, they relay and intensify the death stimuli either over direct initiation of the effector caspases-3, -6 and -7 that are commonly seen

in lymphocytes or by encouraging BID engagement of BAX and BAK to trigger the intrinsic apoptotic pathway, an event that is usually seen in type 2 cells for example hepatocytes.

The Intrinsic Pathway

This pathway is regulated by Bcl-2 family of proteins. These proteins modulate apoptosis and are often overproduced in several malignancies even in the lacking of the existence of the chromosomal translocations. Amplified expression of Bcl-2 leads to resistance of cancer cells to chemotherapeutic drugs as well as radiation therapy (Zhang *et al*., 2021). Accordingly, the decrease of Bcl-2 expression may increase apoptotic responses to anticancer drugs. Moreover, the overexpression of Bcl-2 can end up in buildup of cells in the G0 phase of cell cycle and make up to chemoresistance.

The Bcl-2 family is made up of proapoptotic members such as Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and antiapoptotic members such Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1. Antiapoptotic Bcl-2 members work to repress apoptosis by hindering the release of cytochrome-C, while proapoptotic members serve as apoptosis promoters. These events are mainly dependent on the equilibrium between Bcl-2 and Bax than on the quantity of Bcl-2 alone. As a result of a death signal, proapoptotic proteins are subjected to posttranslational modifications which include dephosphorylation and cleavage that end up in their activation and translocation to the mitochondria thus initiate apoptosis. All BH3-molecules need multidomain BH3 proteins; Bax and Bak to create their intrinsic proapoptotic activity (Alabi *et al., 2019*). In reciprocation to apoptotic stimuli, the outer mitochondrial membrane turns out to be permeable causing the liberation of cytochrome-C and second mitochondria-derived catalyst of caspase, which is also termed as direct IAP-binding protein with low pI. Once the cytochrome-C is released in the cytosol it interacts with Apaf-1 activating caspase-9 proenzymes. Active caspase-9 subsequently triggers caspase-3, which in turn initiates the rest of the caspase cascade leading to apoptosis. Activated caspases cause the cleavage of nuclear lamin and the breakdown of the nucleus through caspase-3 (Orchel *et al.,* 2021).

Cimicifuga Racemosa

Black cohosh (*Cimicifuga racemosa*, CR), is a flowering plant from Ranunculaceae family (Salari *et al.,* 2021). It is commonly found in eastern part of North America and the far south of Ontario. This plant often grows in different forest environments but is majorly found in small woodland openings. Its rhizomes have been in use as traditional medicine by Native Americans in relieving gynecological conditions such as menopause symptoms (Kenda *et al.,* 2021). It has been effective in treating sore throat, uterine prolapse, toothache and aphtha, rheumatism and diarrhea among other diseases. In like manner it has worked as an alternate medicine in hormone replacement therapy (HRT) with estrogens and in lessening the intensity and frequency with which hot flushes occur (He *et al.,* 2017). This is because it has inhibitory agents. Its extract, has proved to relieve menopausal symptoms as well as arthritis. In the recent research carried out on breast cancer cell cultures, the application of CR revealed to have an anti-proliferative activity.

MATERIALS AND METHODOLOGY

I. Extraction of bioactive constituents from rhizome of *Cimicifuga racemosa* using maceration

Material Required

Collection of Plant material: The plant material was procured from Vital herbs, Z-26/27 Commercial Enclave Mohan Garden Uttam Nagar New Delhi- 110059**.** They provided us an authentication certificate.

Reagent:

100g of Ground *Cimicifuga racemose,* Ethyl Acetate, magnetic stirrer*,* round bottomed volumetric flasks, Filter papers, Petri plate, Spatula, Petri dish and Silica Beads

Procedure:

100g of ground rhizome of *Cimicifuga racemose* was placed on a round bottomed volumetric flask. The ground rhizome was considered because it has an increased surface area which allows proper mixing with solvent. Ethyl Acetate was then added to the Round bottomed volumetric flask containing the ground rhizome. Two silica beads were then added onto the flask and the flask was covered using cotton wool and foil to prevent Ethyl acetate from evaporating. Ethyl Acetate was preferred solvent because it is a non-polar solvent and is therefore able to dissolve both polar and non-polar molecules. The flask was then placed on the shaker for three days to ensure occasional shaking was provided for increased diffusion of Ethyl Acetate into the ground *Cimicifuga racemosa*.

After 3 days, the concentrated solution is filtered by use of filter papers. The obtained filtered out liquid was placed on the petri plate and left overnight to dry. The solid residue which is termed as marc of this extraction was preserved for more rounds of extraction yields. The occluded solution, the filtrate after it dries consists of the *Cimicifuga racemosa* bioactive constituents. The dried sample was collected using a spatula and kept in a petri plate. The Petri plate with the sample was stored at 4 degrees Celsius in the refrigerator to ensure the active compounds are protected from disintegration.

2. Evaluation of antioxidant potential of *Cimicifuga racemosa* rhizome extract by FRAP Assay

Material and Equipment Required:

Acetate Buffer, 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), Hydrochloric acid (HCl) Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), 1mg of *Cimicifuga racemosa*

Procedure:

a. Preparation of the reagents to be used in this protocol:

a) Acetate buffer 300 mmol/L **:**

- Weighed 0.19 g of sodium acetate in a beaker of appropriate size
- Dissolved the salt with 10 ml of autoclaved distilled water
- Transferred the solution to a 100 ml volumetric flask
- Added slowly 1.6 ml of glacial acetic acid to the volumetric flask
- Using ultrapure water, completed the solution's volume to reach 100 ml
- Used a pH sensor to evaluate the solution and used drops of glacial acetic acid to maintain the pH to 3.6
- Stored the solution in an amber flask at 8 °C

b) 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) 10 millimolar (mM) **+ Hydrochloric acid (HCl)** 40 millimolar (mM):

- Prepared a solution of HCl 40 millimolar (mM) using HCl 37 %
- Filled a 10 ml volumetric flask with 5 ml of autoclaved distilled water
- Added 40 µL of HCl 37 % to the volumetric flask
- Used autoclaved distilled water to complete the solution's volume to reach 10 ml. (This solution is suited for use for 2 days)
  Weighed 0.031 g of TPTZ in a beaker of appropriate size
- Added 8 ml of HCl 40 millimolar (mM)
- Transferred the solution to a 10 ml volumetric flask
  Using HCl 40 millimolar (mM) the solution's volume was made up to 10 ml

- Stored this solution in an amber flask at 8 °C

c) Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) 20 millimolar (mM):

- Weighed 0.0541 g of ferric chloride Iron(III) chloride hexahydrate
- Transferred the Iron(III) chloride hexahydrate to a beaker of appropriate size
- Added 5 ml of autoclaved distilled water to the beaker to dissolve the salt
- Transferred the solution to a 10 ml volumetric flask
- Used autoclaved distilled water to make up the solution's volume to reach 10 ml
- This solution was prepared on the day of the experiment

2.2 Preparation of FRAP Working solution:

Mixed the following reagents in a proportion of 10:1:1

- Acetate buffer 20 ml
- TPTZ solution 2 ml
- Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) 2 ml

2.3 Sample preparation:

- Weighed 1mg of *Cimicifuga racemosa*
- Added 1ml of Methanol to dissolve the extract
- Prepared 4 different concentration of extract as in the table below using normality equation (*N1V1=N2V2*)

Concentration	Extract from 1mg of *Cimicifuga racemosa* dissolved in 1ml of Methanol	Solvent
100 µ/ml	10 µL	490 µL
250 µ/ml	25 µL	475 µL
500 µ/ml	50 µL	450 µL
1000 µ/ml	100 µL	400 µL

2.4 FRAP Assay procedure;

- Prepared (12) 1ml microtubes to be used to store the samples with the correct information on extract concentration
- 12 microtubes were used to handle 10 µL of each of the four concentrations in triplicates.
- One control microtube for each microtube was maintained using Ascorbic acid of exact concentration as the *Cimicifuga racemosa* rhizome extract.
- 1ml of FRAP working solution was added to each of the 12 microtubes containing different concentrations.
- The microtubes were then incubated for 20 minutes
- Absorbance was then taken at 593 nm

3. **Investigation of the efficacy of *Cimicifuga racemosa* rhizome extract against cervical cancer HeLa cells.**

1. **Material and method required**

Human cervical cancer HeLa cells (HPV 18+) were obtained from NCCS (National Centre for Cell Sciences, Pune). The cell culture was then performed under standard culture

conditions as well as experienced trained guidance. Cell lines were shipped as growing cultures in flasks containing standard shipping media and the cryopreserved cells were revived by cell thawing under standard conditions on dry ice in cryopreservation vials at ambient temperature. Upon receipt of frozen cells, the cells were immediately revived by thawing, removing the DMSO and placing them into culture. When this stage is not possible, the cells are stored in liquid nitrogen vapor (below -149°C), to prevent the cells' viability from declining rapidly.

1.1 CELL THAWING

Materials required:

Cryo vial containing frozen cells, complete growth medium, and tissue - culture treated flasks, 70 % ethanol, petri plate, pipette (1ml), protective gloves and discarder.

Procedure:

- Retrieved a vial of frozen cells from the vapor-phase of liquid nitrogen freezer and transferred to water bath.
- Thawed the vial by gentle agitation in a water bath at 37°C or the normal growth temperature for the HeLa cell line.
- Thawing was done for 2-3 minutes until all ice crystals had completely melted.
- Removed the vial from the water bath, blotted them to dryness and swabbed them thoroughly with 70 % ethanol then transferred the vial to a laminar flow hood.

Prior to experiments the preliminary cell culture steps were done with high precaution as follows;

2. MEDIA PREPARATION

2.1 Media preparation for HeLa cell line:

Materials required:

Glass bottle, syringe filter, powdered media (MEM) (catalogue no. AT006-5L) and sodium bicarbonate, 10 % FBS (fetal bovine serum), 1 % antibiotic and pH meter.

Procedure:

- Desired amount of powdered media, L-Glutamine and sodium bicarbonate was weighed.
- Dissolved the powdered media in desired volume of autoclaved and UV- treated double distilled water.
- Added 10% FBS (growth supplement).
- Added 1% antibiotic to avoid the microbial contamination.
- Filtered the media by 0.22-micron syringe filter into a new bottle and the media is ready for use.

The composition of media for different volumes were as follows;

Compound	1000 ml	200 ml	100 ml	50 ml
Media	9.8gm	1.96gm	0.98gm	0.49gm
Sodium bicarbonate	1.5gm	0.3gm	0.37gm	0.185gm

Storage

- Stored the media at 0-5°C.
- Deterioration of media was constantly marked out by checking color change which is as a result of pH change and precipitation of its components.

2.2 Culture Planting

Materials required: FBS (Fetal bovine serum), antibiotic, measuring cylinder, 0.22 µm syringe filter and T25 and T75 flask.

Procedure:

- To the prepared media 10% FBS and 1% antibiotic were added to make it complete media. (For example, for 100ml of complete media 10mlof FBS and 1ml of antibiotic were added).
- Finally, it was filtered through 0.22 µ syringe filter and it was ready for use in cell culture.

2.3 PASSAGING OF CELLS

Materials required:

T-25 culture flasks, 1X PBS, 0.25% Trypsin – EDTA, pipette discarder, complete growth medium and hemocytometer.

Procedure: The population doubling time (POT) of HeLa cells was approximately 29 h. Cells were sub-cultured when the HeLa cells were at 80-90% confluence or when the cells density reached an average of 1.5 x 10^8 viable cell/cm^2.

- T-25 culture flask was examined carefully for signs of contamination or deterioration and placed in laminar hood.
- The cell culture medium was discarded from the flask and the cell monolayer were rinsed with sterile 1×PBS and then removed.
- 1-2 ml of 0.25 % Trypsin - EDTA (cell - dissociation reagent) was added to the culture flask at the appropriate temperature.
- The cells were examined under inverted microscope to confirm their complete detachment.
- Once the cells appeared to be detached, 6-8 ml of complete growth medium was added to the cell suspension to inactivate the trypsin.
- Then added 5-10 ml of fresh culture medium to a new T-25 culture flask and equilibrated this medium to the appropriate pH and temperature.
- Counted the cells in suspension and determined their viability/divided them according to 1:2 split ratios and dispensed them into the medium of the newly prepared flask.
- Incubated the flask back into CO2 incubator at 37°C.

2.4 CELL COUNTING

Materials Required:

Hemocytometer, Trypan blue dye, Trypsin-EDTA, cell suspension, cover slips, pipettes, 70% ethanol, falcon tube and fresh complete media.

Procedure:

- Cell culture of a T-25 flask was trypsinized by using 0.25% trypsin-EDTA and transferred into a falcon tube then centrifuged at 1000 rpm for 1 to 2 minutes at 4°C.
- The supernatant was then discarded while the crystal was suspended into 1 ml of complete media.
- Then 1:10 dilution of the cell suspension was prepared in the complete media (20µl of the cell suspension and 180µl complete media).
- Staining of the cells was done by adding 10µl of trypan blue dye to the 10µl of the cell suspension.
- Then cleaned, thoroughly dried and assembled the hemocytometer with the cover slip.

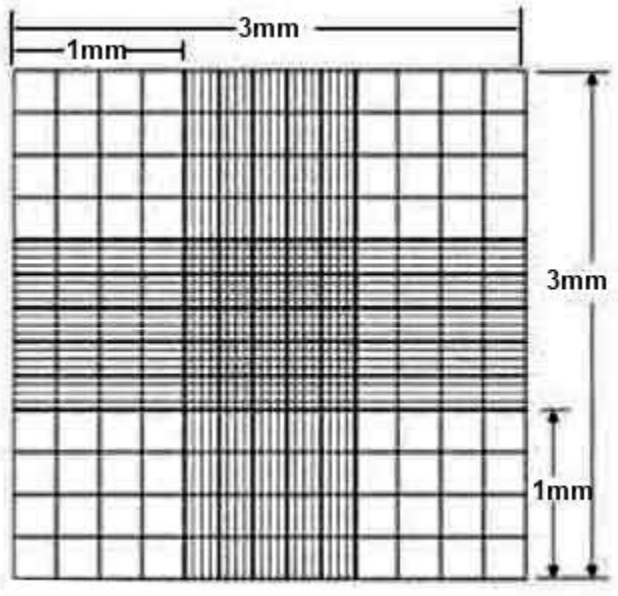

***Figure 8*:** Hemocytometer grid with Neubauer ruling

- The 20µl trypan blue cell suspension was transferred to the edge of each of the two counting chambers. The cell suspension was allowed to be drawn into the counting chamber by capillary action.
- The hemocytometer was then placed under an inverted microscope and viewed the cells at 100X magnification.
- All the cells were counted in each corner square (non-viable cells were stained blue and viable cells were remarked colorless).
- The subsequent cell concentration per ml and the total number of cells were determined by using the following formula.

Total cell/ml = Total cells counted X Dilution factor/Number of squares X 10,000 cells/ml

2.5 Morphological Analysis by Phase Contrast Microscopy

Material required:

Complete fresh media, Trypsin-EDTA, 100% confluent T-25 flask, falcon tube, centrifuge, pipettes, cell counter, 6-well plate, discarder, DMSO, carvacrol, 5FU and combine (carvacrol+5FU).2

Procedure:

- 96-well plate was used for morphological analysis by phase contrast microscopy. Each experiment was done in triplicate.
- Using aseptic techniques, HeLa cells were trypsinized from T-25 flask.
- Re-suspended the cells at 5 X 10^3 per ml.
- Plated out, in triplicate, 100µl of the dilution into wells of a 96-well plate.
- Included three control wells of medium to provide the blanks for absorbance readings.
- Incubated the cells under conditions appropriate for the cell line for 24 h.
- After incubation the cells were treated with different concentrations (800µg/ml to 1.6mg/ml) concentration of *Cimicifuga racemosa* rhizome ethyl-acetate extract.
- Incubated the treated cells for 24 h

- Used an inverted fluorescent microscope (Nikon ECLIPSE TiS, Japan) to visualize intracellular fluorescence of cells and to capture images.

2.6 MEASUREMENT OF CELL VIABILITY BY MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide]

Material required:

Complete fresh media, Trypsin-EDTA, 100% confluent T-25 flask, falcon tube, centrifuge, pipettes, cell counter, 96-well plate, discarder, DMSO, drugs, MTT dye and silver foil.

Procedure:

- 96-well plate was used for MTT assay. Each experiment was done in triplicate.
- Using aseptic techniques, HeLa cells were trypsinized from T-25 flask.
- Re-suspended the cells at 5 X 10^3 per ml.
- Plated out, in triplicate, 100µl of the dilution into wells of a 96-well plate.
- Included three control wells of medium only to provide the blanks for absorbance readings.
- Incubated the cells under conditions appropriate for the cell line for 24 h.
- After incubation cells were treated with different concentrations (100µg/ml to 400µg/ml) concentration of *Cimicifuga racemose* for 24 h.
- Added 10µl of MTT reagent (equal to 10% of the total well volume) to each well, including blanks.
- Returned the plate to cell culture incubator for 3 h (a purple precipitate was clearly visible under the microscope after this incubation period).
- Added 100µl of MTT solubilization solution which is dimethylsulfoxide (DMSO) amount equal to the original total volume was used in all wells, including blanks.
- Removed plate cover and measured the absorbance in each well, including the blanks, at 595nm and 690nm in a micro plate reader.

RESULTS

1. Antioxidant Activity of *Cimicifuga racemosa* Rhizome Extract by FRAP Assay

After incubating the 12 microtubes containing 10 µL of each of the four concentrations of *Cimicifuga racemosa* rhizome extract for 20 minutes, a dense blue color was observed. The absorbance of the incubated triplicates was then taken at 593 nm using UV-visible spectrophotometry and the readings were generated as the standard curve below .

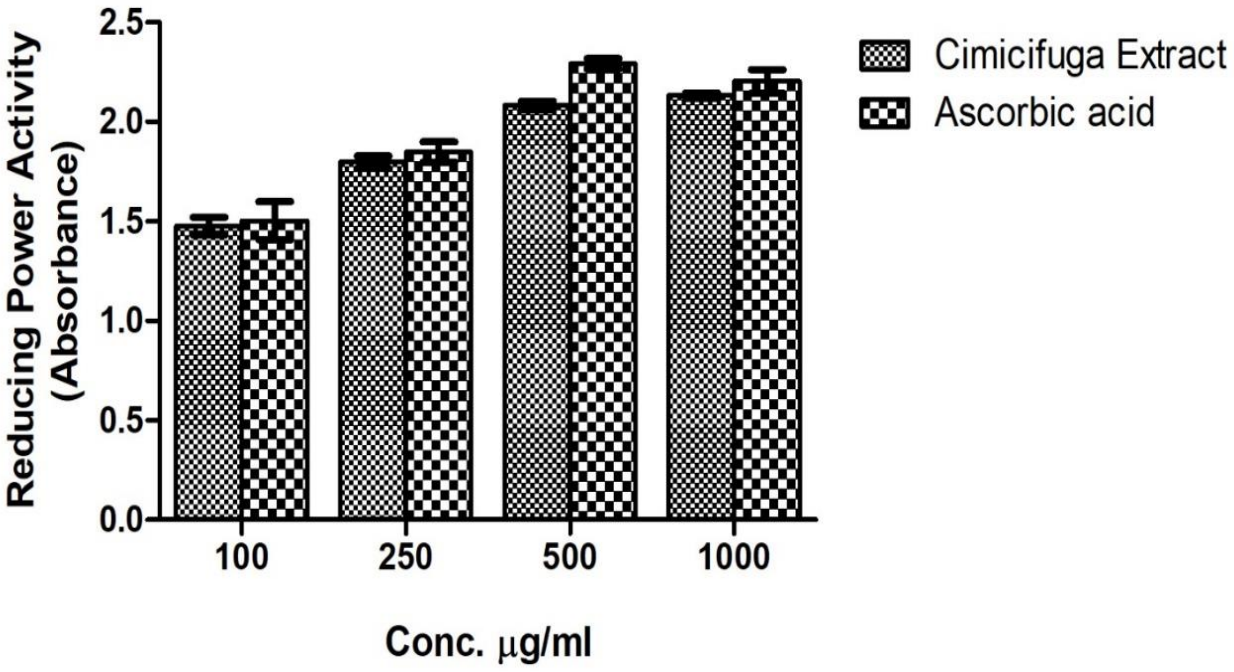

Figure 9: *Analysis of Antioxidant activity of Cimicifuga racemosa in relation to the standard Ascorbic acid.*

2. Morphological Changes in cervical Cancer Cells induced by *Cimicifuga racemosa* rhizome extract

Phase-contrast microscopic analysis of HeLa cells treated with different concentration of *Cimicifuga racemosa* rhizome extract (100µg/ml to 400µg/ml) revealed significant morphological changes when compared to the control. The 400µg/ml treated well of cervical cell line showed much morphological difference when compared to 100µg/ml, 200µg/ml and 300µg/ml treated wells. The morphological difference was accounted for by more retraction of cellular processes and cell shrinkage according to the different concentration of *Cimicifuga racemosa* rhizome extract as opposed to the cells in the control well that remained spread with a flattened morphology.

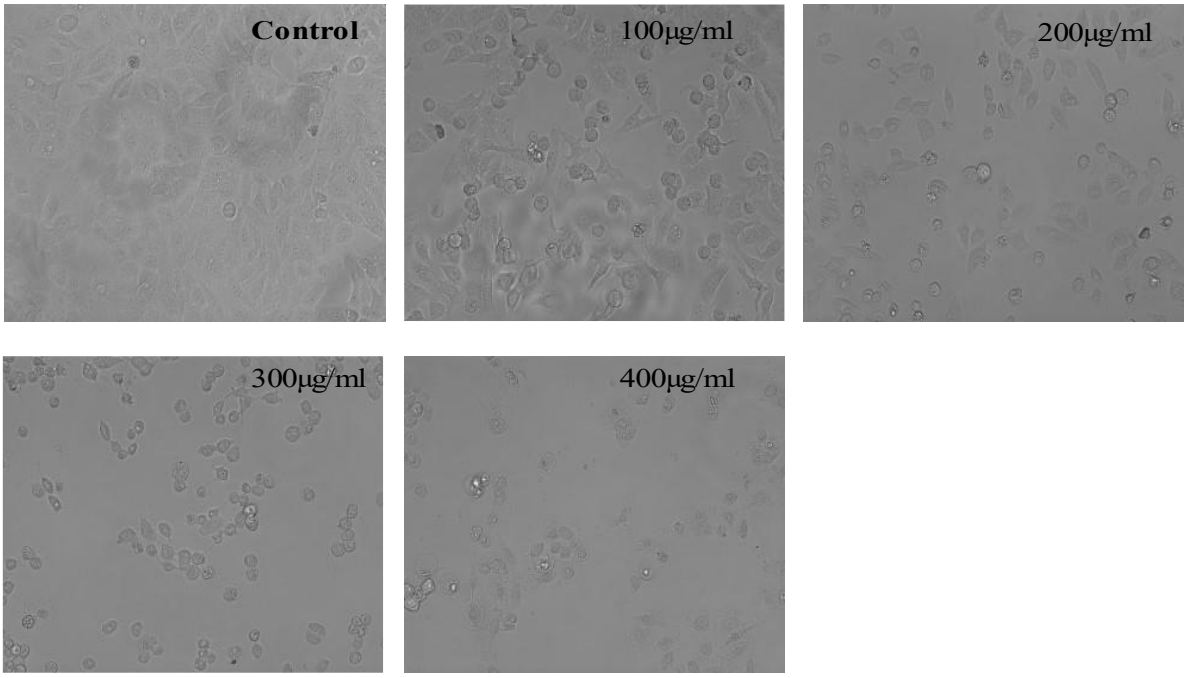

Figure 10: *Phase-contrast photomicrograph of HeLa cells treated with different concentration of Cimicifuga racemosa rhizome extract for 24 h.*

3. Cell viability effect of *Cimicifuga racemosa* rhizome extract on CC HeLa cells

To find out the cytotoxicity of experimental doses of *Cimicifuga racemosa* rhizome extract**,** MTT assay and assessment of cell morphology were performed on HeLa cells. Cell viability data showed that after 24 h of treatment, percent cell viability of HeLa cell as compared to control was 89.13%, 76.85%, 43.15% and 30.25% at 100µg/ml, 200 µg/ml, 300µg/ml and 400µg/ml dose of CR extract respectively. This data was found to be statistically significant.

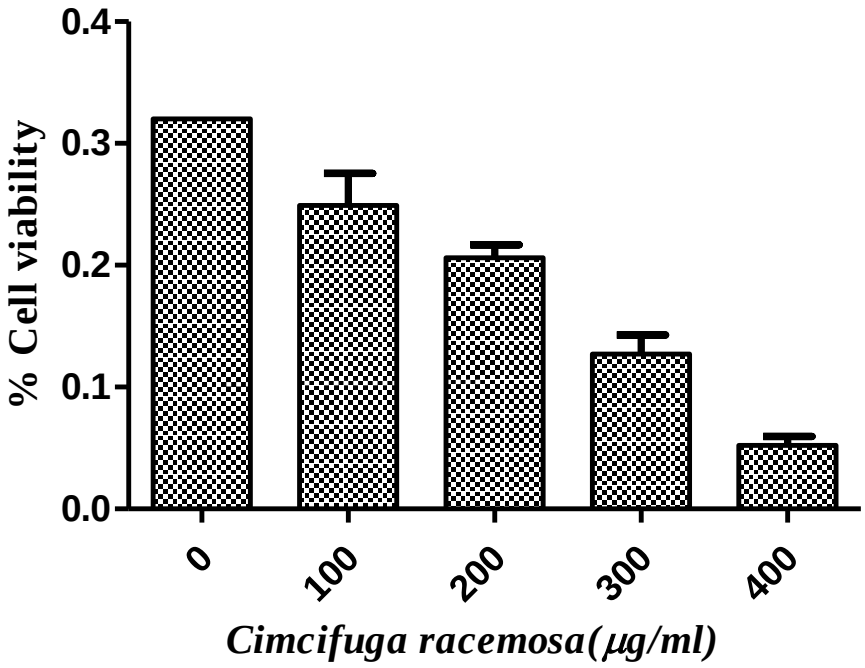

Figure 11: *Effect of Cimicifuga racemosa rhizome extract on proliferation of cervical cancer cell for 24 h assessed by MTT assay. The results represented are the Mean ± S.E.M of three independent experiment performed in triplicates.*

4. Determination of IC_{50} Value using the MTT Assay results

To characterize the concentration at which *Cimicifuga racemosa* rhizome extract utilized half of its maximal inhibitory effect on HeLa cells, MTT Assay results were used to generate $IC_{50\ value}$.

This value classically illustrates the effectiveness of *Cimicifuga racemosa* rhizome extract in inhibiting the proliferation of HeLa cells.

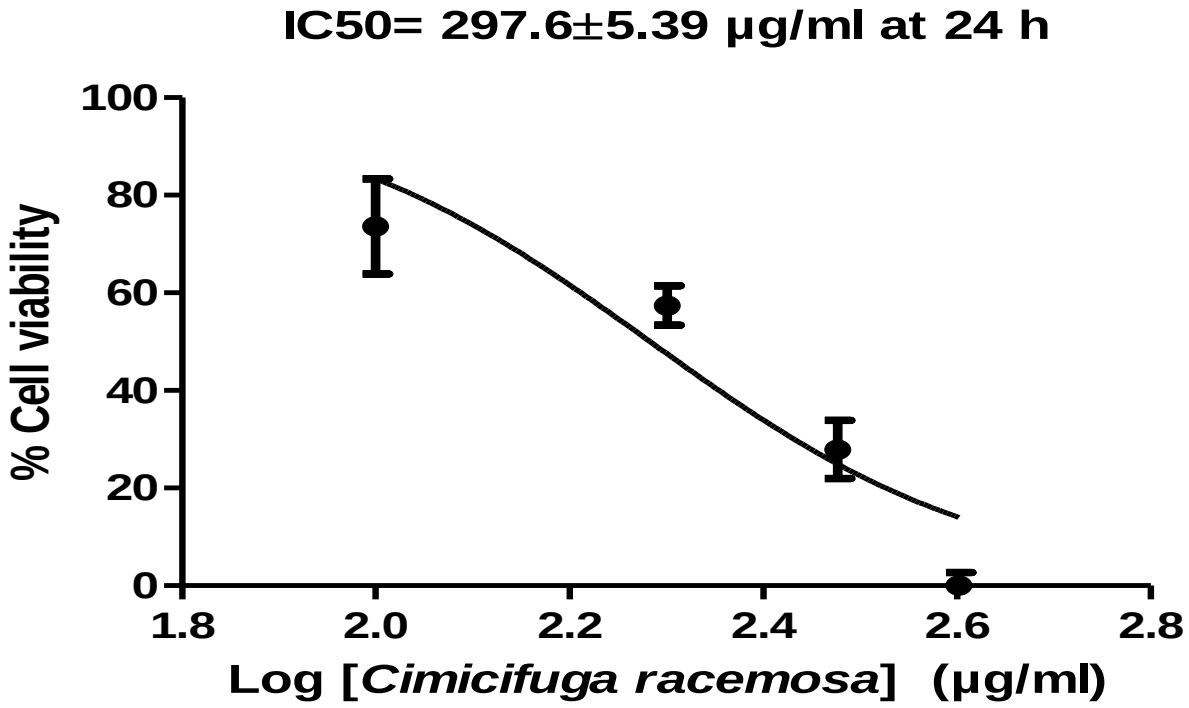

Figure 12: *Effectiveness of Cimicifuga racemosa rhizome extract in inhibiting the cell growth of HeLa cells. The $IC_{50\ Value}$ of Cimicifuga racemosa rhizome extract was 297.6 ±5.39 µg/ml at 24 h.*

DISCUSSION

FRAP Assay proved that CR rhizome extract has significant levels of antioxidants when compared to the standard, Ascorbic acid.

The CC HeLa cells treated with different concentration of CR rhizome extract showed significant effect morphologically as the CR concentration were increased successively per well. This effect was attributed to the high antioxidant levels among other bioactive agents in CR rhizome extract.

The MTT Assay performed on the CC HeLa cells that had been treated with different concentrations of CR rhizome extract for 24 h showed reduced cell viability and proliferation successively per well as the CR concentration increased.

CR rhizome extract exhibited its half of maximal inhibitory effect on HeLa cells at 297.6 ±5.39 µg/ml at 24 h. This value showcases the efficiency of the extract in reducing the viability of HeLa cells.

CONCLUSION

In conclusion, the present study provides novel understanding of the mechanism of antiproliferative and apoptotic action of CR rhizome extract against human Cervical cancer cells which has never been reported in any research paper.

The results show a link between antiproliferative, apoptotic induction and cell death in CC HeLa cells due to nuclear condensation, cellular shrinkage and the induction of ROS-mediated mitochondrial membrane depolarization.

This study confirmed the potential of *Cimicifuga racemosa* rhizome extract as a chemo-preventive agent in human cervical cancer HeLa cells with the involvement of apoptosis.

RECOMMENDATION

Further studies need to be carried out to fully identify the mechanism by which *Cimicifuga racemosa* rhizome extract manages to induce antiproliferative and apoptosis on human Cervical Cancer HeLa cells.

Cimicifuga racemosa rhizome extract has significant effect on cancer cells and it can potentially provide valuable efficacy. Therefore, it is ideal to be used during drug development.

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