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PRELIMINARY PHYTOCHEMICAL AND IN-VITRO PHARMACOLOGICAL SCREENING OF TECOMARIA CAPENSIS ONCOLON CANCER CELL LINE (HCT15)

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ABSTRACT

Introduction: Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy.

KEY WORDS: Tecomaria capensis, ethyl acetate, colon cancer.

The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita.

Cancer, also known as a malignant tumor or malignant neoplasm, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[15,16] Not all tumors are cancerous; benign tumors do not spread to other parts of the body Possible signs and symptoms include: a new lump, abnormal bleeding, a prolonged cough, unexplained weight loss, and a change in bowel movements among others.[17] While these symptoms may indicate cancer, they may also occur due to other issues. There are over 100 different known cancers that affect humans.

Tecomaria capensis is an evergreen scrambler to small tree with a roundish crown. Bark pale brown, lenti celled with longitudinal furrows on old stems. Leaves opposite, unevenly compound, up to 13 cm long, with 2-5 pairs of leaflets, terminal leaflet largest, margins coarsely toothed, glossy green above. Fruit a narrow, flat pod-like capsule up to 13 cm long.

Seeds with large papery wings. There are 3 garden cultivars; “coccinea” with light red flowers on a bushy plant, “lutea” with bright yellow flowers on a spreading bush and “salmonii” with salmon-coloured flowers. The genus Tecomaria is monotypic and has affinities with Tecoma.

Biology

The cape honeysuckle is dioecious and evergreen; usually flowering after rains from June-November and fruiting from October-February. Pollinated by birds and insects. Calyx 5-lobed, much shorter than corollatube. Corolla bilabiate, tube curved, narrowly funnel-shaped; one lip 2-lobed; all lobes elliptic, obtuse. Stamens didynamous, inserted in lower part of corolla-tube, exserted; filaments terete; anthers 2- thecous with thecae at length separating. Style terete, exserted, with elliptic, 2-lobed stigma.

Aim of the Study

The main Aim of the study is to screening the phytochemical and in-vitro pharmacological study of TECOMARIA CAPENSIS on Colon Cancer cell line (HCT 15).

METHODOLOGY

The techniques commonly used in the field of phytochemistry are extraction, isolation, and structural elucidation of natural products, as well as chromatographic techniques. The solvent extraction of any botanical materials may yield very less quantity of volatile oils and a large yield of non volatile components like resins, pigments, waxes and fatty acids.

MTT Assay Method:[235]

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high through put screening. Measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is accurate but it is also time-consuming and

involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

The MTT method of cell determination is most useful when cultures are prepared in multi well plates. For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells.

RESULTS AND DISCUSSION

In-vitro cytotoxic activity of *Tecomaria capensis* ethyl acetate extract various concentration against Colon cancer cell line (HCT15) cancer cell lines were studied using MTT assay. Antitumour activity of *Tecomaria capensis* ethyl acetate extractat various concentrations against Colon cancer cell line (HCT15) cancer cell lines was represented in Figure 22. With increase in concentration of *Tecomaria capensis* ethyl acetate extractfrom 25, 50, 100, 250, 500 µg/ml. documents reduced percentage of cell viability respectively. Then the percentage of cell density has been decreased evident the cell death.

Summary & Conclusion

The results described here clearly confirmed the anti-cancer properties of *Tecomaria capensis* ethyl acetate extract.Present study infers that the *Tecomaria capensis* ethyl acetate extract exhibit effective antitumor activity and seems to have no side effects. They are less cost effective, easy in production andpurification. In future it can be recommended to the patients as a effective therapeutic tool in form of food or drug. Further research need to be explored to study the bioactive compounds of *Tecomaria capensis* ethyl acetate extract and for the successful implication of them as a potent therapeutic tool against cancer.

1. INTRODUCTION

1.1 Introduction To Herbal Medicine

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. This canbe achieved by judicious product identification based on diseases found in the developed world for which no medicine or only palliative therapy is available; such herbal medicines will find speedy access into those countries.[1]

The basic requirements for gaining entry into developed countries include

1. Well-documented traditional use
2. Single plant medicines,
3. Medicinal plants free from pesticides, heavy metals
4. Standardization based on chemical and activityprofile
5. Safety and stability.

However, mode of action studies in animals and efficacy in human will also be supportive.Such scientifically generated data will project herbal medicine in a proper perspective and help in sustained global market.

Evidence of Herbal medicine: The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plantpreparations for therapy.

The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita.

Choice of herbal medicine

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser

side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believedto have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc., for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials.

Herbal medicine scenario in India

The turnover of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of Ayurveda, Unani and Siddha systems of medicine is about $ 1billion with a meagre export of about $ 80 million. Psyllium seeds and husk, castor oil and opium extract alone account for 60% of the exports. 80% of the exports to developed countries are of crude drugs and not finished formulations leading to low revenue for the country. India is one of the 12 mega biodiversity centre's having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000–18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms.[2]

Role of WHO in herbal medicine

Ill health or disease is brought about by an imbalance or disequilibrium of man in his total ecological system and not only by the causative agent and pathologenic evolution (WHO[3]). In 1991 WHO developed guidelines for the assessment of herbal medicine,[4] and the same were ratified by the 6th International Conference of Drug Regulatory Authorities held at Ottawa in the same year. The salient features of WHO guidelines are: (i) Quality assessment: Crude plant material; Plant preparation; Finished product (ii) Stability: Shelf life (iii) Safety assessment: Documentation of safety based on experience or/and; Toxicology studies (iv) Assessment of efficacy: Documented evidence of traditional use or/and; Activity determination (animals, human).

Safe and Unsafe Herbs

Some herbal medicines are considered to be comparatively safe. These include Feverfew (*Tanacetum parthenium*) used in the prophylaxis of migraine headaches, treatment of fever, menstrual problems, asthma, dermatitis and arthritis. Garlic (Allium *sativum*) is used in hyperlipoproteinemia and prevents arteriosclerosis. Other herbs are Ginkgo (*Ginkgo biloba*), saw palmetto (*Sereno arepens*), Asian ginseng (*Panax ginseng*), St. Jhon'sWort (*Hypericum perforatum*), Valerian (*valeriana officinalis*) and Ginger (*Zingiber officinale*). They do possess some therapeutic value and are increasingly the subject of clinical trials in which their efficacy tolerability and safety are being compared with allopathic medicines. Some herbs possess constituent with toxic potential. These include *Acorus calamus* (mutagenic and carcinogenic), *Anthoxanthum odoratum* (hepatotoxic), *Artemisia absinthium* (neurotoxic), *Callilepis laureola* (nephrotoxic and hepatotoxic), *Conium maculae* (teratogenic), *Croton tiglium* (purge and tumor promoting), *Stephania spp.* (CNS depressant and hepatotoxic), *Erythroxylum spp*. (Psychotism) and *Glycyrrhiza* (pseudoaldosteronism) considered carcinogenic, Borage (*Borago officinalis*), Comfery (*symphy spp.*) and Life root (*Scenecio aureus*). Unfortunately, checking the label herbal package is not always sufficient to exclude the potential contaminations because some of the currently available preparations are contaminated intentionally or accidentally, or by potential herbal or non-herbal substance and sometimes with microorganisms.[5]

Fingerprinting/Chemo profiling

Fingerprinting in essence is chemo profiling while establishing a characteristic chemical pattern for a plant material or its cut or fraction of extract. It is important to understand that a plant extract consists of establishment of chemical compounds. These include the primary metabolites, secondary metabolites and inorganic metals. Primary metabolites are components of carbohydrates, proteins, lipids which are essential for the plant physiology. Secondary metabolites are metabolites which are not essential for plant physiology and are formed as byproducts in the biochemical reactions. These include very interesting and useful compounds like alkaloids, flavonoids, coumarins, anthocyanins, etc. Many modern drugs have came from secondary metabolites like morphine from opium, serpentine from Rauwolfia, vinblastine and vincristine from *Vinca rosea*. Infact this is the point of divergence for modern system of medicine and classical system of medicine like Ayurveda with the former laying emphasis on compounds. However coming to the main line, one can utilize these secondary metabolites for the identification of plant material as our knowledge of chemistry advanced sufficiently and through sophisticated techniques we can measure these compounds qualitatively and quantitatively. But the catch lies here for two factors, First if in a plant material we can measure the presence of unique secondary metabolites it is not sufficient to decide certainly that the plant material is of the desired quality. Second important factor is that there are no data for characterizing compounds even for phytochemical studied materials. The herbal drug industry is looking into number of these characterizing compounds obtained from specific plant materials.[6]

Multidisciplinary Approaches

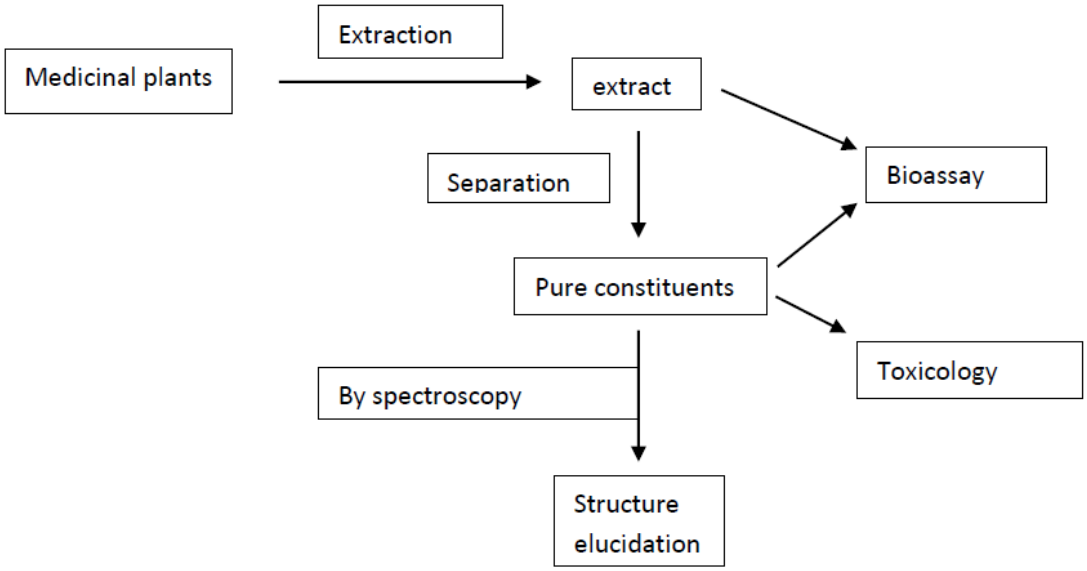


Figure 1: Pathway From Plant To Pure Bioactive Constituents Standardisation And Quality Evaluation Parameters Of Herbal Drugs.

For safe and effective use of herbal drugs, consistency in composition and biological activity are essential. However, herbal drugs frequently fail to meet this standard due to some problems such as

- Difficulties in identification of plants
- Genetic variability
- Variations in growing conditions
- Diversity in harvesting procedures and processing of extracts
- Lack of knowledge about active pharmacological principles

Batch-to-batch consistency can be ensured by performing standardization of herbal products with the help of chromatographic techniques and marker compounds. The lack of standardization of herbal drugs would be a serious problem for a researcher as he would not be able to rely on commercially available herbal products for his research study. The standardization and quality evaluation parameter of herbal drugs follows:

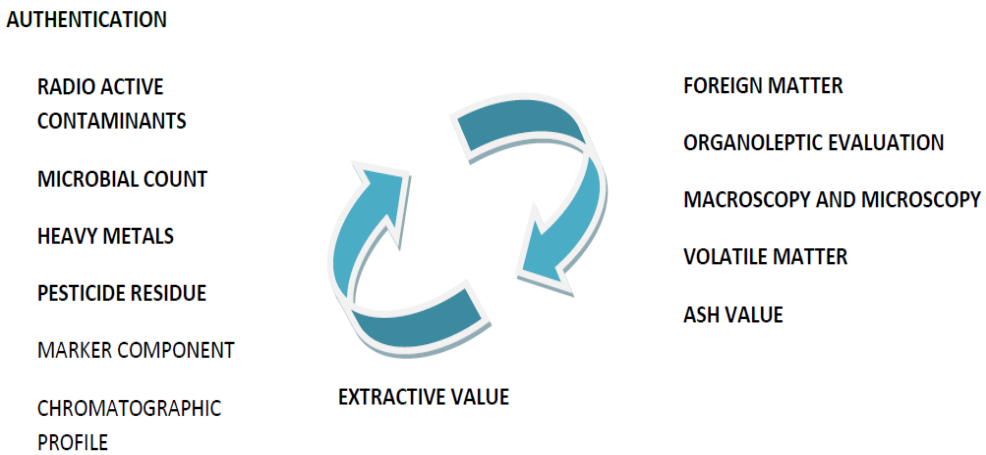


Figure 2: Herbal drugs standardization can be done by performing screening. Screening implies the evaluation of multiple samples in a ritualized fashion using a standardized single technique or tests, which is proportionally more expensive and tedious.

Screening of herbal drugs for biological activity include[7]

- Primary pharmacological screening
- Secondary and tertiary acute pharmacological /toxicological evaluations
- Chronic pharmacological/toxicological evaluations
- Product formulations
- Clinical trials and
- Release in system for therapeutic utilization

Screening Approaches
Three types of screening approaches define herbal drugs,

- Primary
- Secondary
- Tertiary

Primary Screening Past Approach
Phytochemical and chemotherapeutic screening remains the dominant trends for screening programs.

Present Approach
The pharmacological screening can be divided into four styles

Single Technique-Single Goal Screening
This utilizes a single technique aiming at a single goal as the activity of natural product.

Screening using a battery of specific procedures
This utilizes multiple specific tests to define pharmacological activity of a crude drug.

Single technique multiple goals screening
This utilizes multiple observations-single techniques to search for virtually any and all pharmacological activity in a single crude drugs.

Combination of specific and multipurpose procedures:
This includes various specific and multipurpose procedures.

Secondary Screening
Primary screening is always conducted using only minimum amount of carefully authenticated crude drug. If promising activity has been found during primary screening then a sizeable quantity of authenticated plant material is acquired and secondary evaluations organized. Secondary testing is confirmed in another species of laboratory animals, the activity noted in primary screen and should consist of drug/drug interaction experiments.

Teritiary Screening
Teritiary screening is expensive and time consuming. The course taken for a single drug depends on data accumulated during primary and secondary phases of evaluation and open for further research.

All these methods account for a single chemical entity or a group of chemical compounds, but in many plants the activity may be attributed to different types of compounds that act synergistically to show the desired biological activity. Therefore, standardization by chemical methods, although used widely, may not prove to be a complete way of standardization and further need biological standardization.

Biological Assays
Biological assays ensures consistent clinical efficacy of herbal product from batch to batch. The analytical methods including chromatographic evaluation some times are ineffective as they are usually insensitive to the chemical complexities found in crude botanical extracts. The biological potency of the herbal drug is due to not one but a mixture of bioactive plant constituents. The relative properties of a single bioactive compound can vary from batch to batch while the biological activity remains within the desirable limits.

Thus, it is desirable to incorporate bioassay as an additional method of standardization, which in turn becomes an effective quality control methods.

Bioassays are broadly classified as

- General screening bioassay
- Specific bioassay
- Primary screening bioassay

Geeral Screening Bioassay
These are non-selective and indicate biological activity of the herbal drugs.

Primary Screening Bioassay
This helps in identification of bioactive compounds.

Specific Bioassay
It provides specific bioactivity and a large number of herbal drugs have been standardized by using this method.

Reverse Pharmacology
Reverse pharmacology is defined as the science of integrating documented clinical experiences and experimental observations into leads by trans disciplinary exploratory studies and further developing these into drug candidates or formulations through robust preclinical and clinical research.[8] The traditional knowledge inspired reverse pharmacology described here relates to reversing the routine 'laboratory to clinic' progress of discovery pipeline to 'clinics to laboratories'.[9] In this progress 'safety' remains the most important starting point and the efficacy becomes a matter of validation.

Sir Ram Nath Chopra and Gananath Sen laid the foundation of reverse pharmacology of medicinal plants by pursuing clinically documented effects of Ayurvedic drugs.[10] *Rauwolfia serpentina* Benth, was a major discovery via this approach. Sen and Bose in 1931 convinningly demonstrated the antihypertensive and tranquillizing effects of the plant and also observed unique side effects such as depression, extrapyramidal syndrome, gynacomastia and peptic ulcer.[11,12]

Clinical events or phenomenon previously not reported and following the administration of a known or new drug, can provide valuable insights for drug development. Medicinal plants and natural products derived there from have provided many such

serendipitous bedside observations. Historically, several such clinical hits were not often pursued quickly and rigorously by the drug discovery teams. Similarly, research in genomics, proteomics and metabolomics has stimulated discovery of many new entities, which are yet to be pursued for their drug-like activities. A new trans-disciplinary endeavor called Reverse Pharmacology has recently emerged and addresses both these needs. Reverse Pharmacology (RP), designed as an academic discipline to reduce three major bottlenecks of costs, time and toxicity. RP can be perceived to comprise of three phases. First, the experiential phase that include robust documentation of clinical observations of the biodynamic effects of standardized Ayurvedic drugs by meticulous record keeping. Second, the exploratory studies for tolerability, drug-interactions, dose- range finding in ambulant patients of defined subsets of the disease and para clinical studies in relevant *in vitro* and *in vivo* models to evaluate the target-activity. Third phase includes experimental studies, basic and clinical, at several levels of biological organization, to identify and validate the reverse pharmacological correlates of Ayurvedic drug safety and efficacy. The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biology and to optimize safety, efficacy and acceptability of the leads in natural products based on relevant science. In this approach as the candidate travels a reverse path from 'clinics to laboratory' rather than classical 'laboratory to clinics'.[13]

The conventional approach is seeking out new chemical drugs involves identifying the new molecules, testing their efficacy on laboratory animals, and then moving to humans. chemical drug discovery around the world had focused on moving drugs from molecules to mice and then to men. Reverse pharmacology is the alternative and the most suitable approach for efficient discovery (rediscovery) of herbal drugs with very few bottlenecks. In reverse pharmacology we are going the other way- from men to mice to men. Traditional herbal medicine has long been used in clinical practice. Reverse pharmacology is aimed at validating such herbal drugs through modern scientific methods.

Scope

The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biological organization and to optimize safety, efficacy and acceptability of the leads in natural products, based on relevant science.[14]

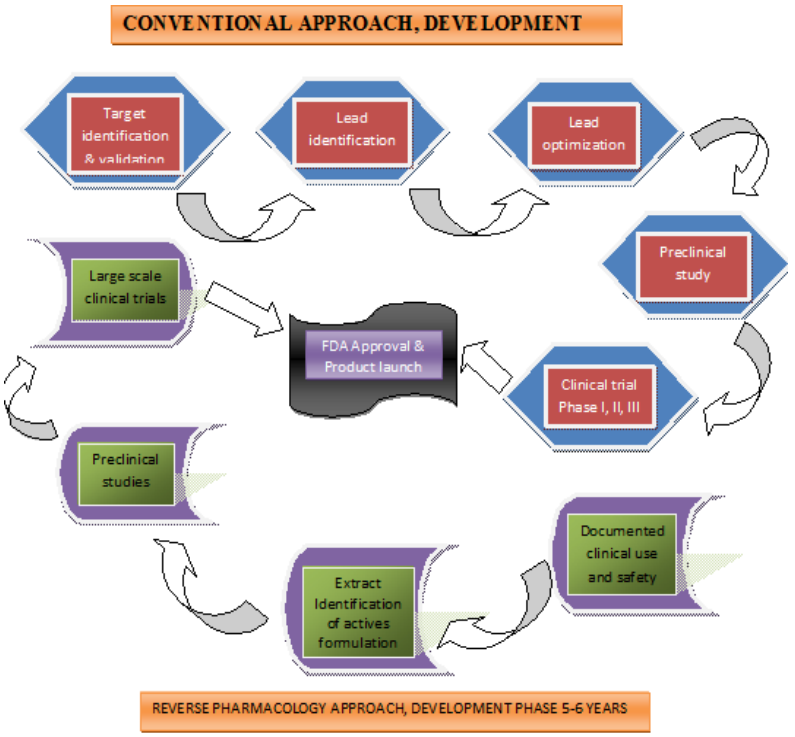


Figure 3: CONVENTIONAL APPROACH VS REVERSE PHARMACOLOGICAL APPROACH

Cancer

Cancer, also known as a malignant tumor or malignant neoplasm, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[15,16] Not all tumors are cancerous; benign tumors do not spread to other parts of the body Possible signs and symptoms include: a new lump, abnormal bleeding, a prolonged cough, unexplained

weight loss, and a change in bowel movements among others.[17] While these symptoms may indicate cancer, they may also occur due to other issues. There are over 100 different known cancers that affect humans.

Tobacco use is the cause of about 22% of cancer deaths.[15] Another 10% is due to obesity, a poor diet, lack of physical activity, and consumption of alcohol.[15][18] Other factors include certain infections, exposure to ionizing radiation, and environmental pollutants.[19] In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C, and human papillomavirus (HPV).[15] These factors act, at least partly, by changing the genes of a cell.[20] Typically many such genetic changes are required before cancer develops.[20] Approximately 5–10% of cancers are due to genetic defects inherited from a person's parents.[21] Cancer can be detected by certain signs and symptoms or screening tests.[15] It is then typically further investigated by medical imaging and confirmed by biopsy.[22]

Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, being vaccinated against certain infectious diseases, not eating too much processed and red meat, and avoiding too much exposure to sunlight.[23][24] Early detection through screening is useful for cervical and colorectal cancer.[25] The benefits of screening in breast cancer are controversial.[25][26] Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy.[15][27] Pain and symptom management are an important part of care. Palliative care is particularly important in those with advanced disease.[15] The chance of survival depends on the type of cancer and extent of disease at the start of treatment.[20] In children under 15 at diagnosis the five-year survival rate in the developed world is on average 80%.[14] For cancer in the United States the average five-year survival rate is 66%.[29]

In 2012 about 14.1 million new cases of cancer occurred globally (not including skin cancer other than melanoma).[20] It caused about 8.2 million deaths or 14.6% of all human deaths.[20][30] The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer, and stomach cancer, and in females, the most common types are breast cancer, colorectal cancer, lung cancer, and cervical cancer.[20] If skin cancer other than melanoma were included in total new cancers each year it would account for around 40% of cases.[21][32] In children, acute lymphoblastic leukaemia and brain tumors are most common except in Africa where non-Hodgkin lymphoma occurs more often.[28] In 2012, about 165,000 children under 15 years of age were diagnosed with cancer. The risk of cancer increases significantly with age and many cancers occur more commonly in developed countries.[20] Rates are increasing as more people live to an old age and as lifestyle changes occur in the developing world.[33] The financial costs of cancer have been estimated at $1.16 trillion US dollars per year as of 2010.[34]

Definitions

Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body.[15][16] They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth, and will often form a mass or lump, but may be distributed diffusely.[35,36]

All tumor cells show the six hallmarks of cancer. These are characteristics that the cancer cells need toproduce a malignant tumor. They include.[37]

- Cell growth and division without the proper signals to do so
- Continuous growth and division even when there are signals telling them to stop
- Avoidance of programmed cell death
- Limitless number of cell divisions
- Promoting blood vessel construction
- Invasion of tissue and formation of metastases.[38]

The progression from normal cells to cells that can form a detectable mass to outright cancer involves multiple steps known as malignant progression.[38,39]

Signs and symptoms

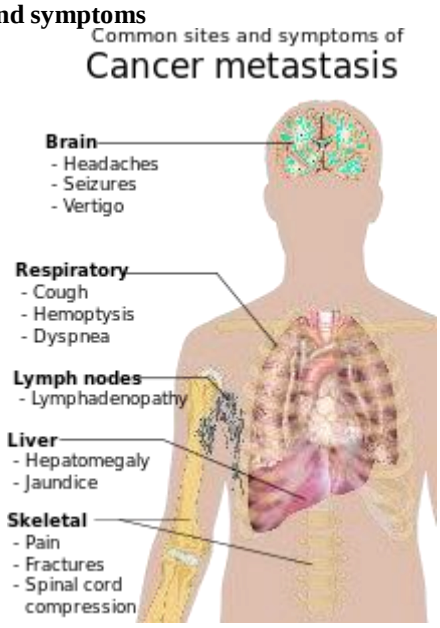


Figure-4: Symptoms of cancer metastasis depend on the location of the tumor.

When cancer begins, it invariably produces no symptoms. Signs and symptoms only appear as the mass continues to grow or ulcerates. The findings that result depend on the type and location of the cancer. Few symptoms are specific, with many of them also frequently occurring in individuals who have other conditions. Cancer is the new "great imitator". Thus, it is

not uncommon for people diagnosed with cancer to have been treated for other diseases, which were assumed to be causing their symptoms.[40]

Local effects

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can cause blockage of the bronchus resulting in cough or pneumonia; esophageal cancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, resulting in changes in bowel habits. Masses in breasts or testicles may be easily felt. Ulceration can cause bleeding that, if it occurs in the lung, will lead to coughing up blood, in the bowels to anemia or rectal bleeding, in the bladder to blood in the urine, and in the uterus to vaginal bleeding. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause a buildup of fluid within the chest or abdomen.[40]

Systemic symptoms

General symptoms occur due to distant effects of the cancer that are not related to direct or metastatic spread. These may include: unintentional weight loss, fever, being excessively tired, and changes to the skin.[41]Hodgkin disease, leukemias, and cancers of the liver or kidney can cause a persistent fever of unknown origin.[40]

Some cancers may cause specific groups of systemic symptoms, termed para neoplastic phenomena. Examples include the appearance of myasthenia gravis in thymoma and clubbing in lung cancer.[40]

Metastasis

Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by blood (haematogenous spread) to distant sites, known as metastasis. When cancer spreads by a haematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as hypothesized in the *soil and seed hypothesis* of cancer metastasis. The symptoms of metastatic cancers depend on the location of the tumor, and can include enlarged lymph nodes (which can be felt or sometimes seen under the skin and are typically hard), enlarged liver or enlarged spleen, which can be felt in the abdomen, pain or fracture of affected bones, and neurological symptoms.[40]

Causes

The great majority of cancers, some 90–95% of cases, are due to environmental factors. The remaining 5–10% are due to inherited genetics.[19]*Environmental*, as used by cancer researchers, means any cause that is not inherited genetically, such as lifestyle, economic and behavioral factors, and not merely pollution.[42] Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants.[19]

It is nearly impossible to prove what caused a cancer in any individual, because most cancers have multiple possible causes. For example, if a person who uses tobacco heavily develops lung cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung cancer as a result of air pollution or radiation, then there is a small chance that the cancer developed because of air pollution or radiation. Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer is generally not a transmissible disease.[43]

Chemicals

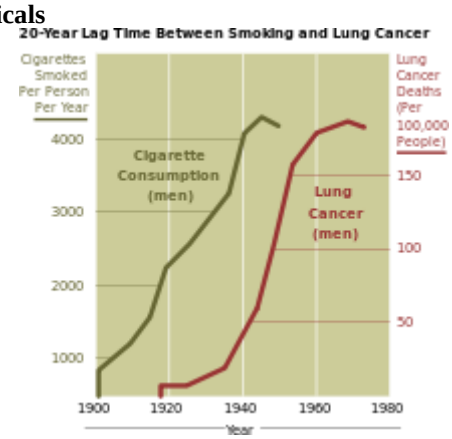


Figure 5: The incidence of lung cancer is highly correlated with smoking.

Exposure to particular substances have been linked to specific types of cancer. These substances are called *carcinogens*. Tobacco smoking, for example, causes 90% of lung cancer.[44] It also causes cancer in the larynx, head, neck, stomach, bladder, kidney, esophagus and pancreas.[45] Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons.[46] Tobacco is responsible for about one in three of all cancer deaths in the developed world,[47] and about one in five worldwide.[32]Lung cancer death rates in the United States have mirrored smoking patterns, with increases in smoking followed by dramatic increases in lung cancer death rates and, more recently, decreases in smoking rates since the 1950s followed by decreases in lung cancer death rates in mensince 1990.[48,49]

In Western Europe, 10% of cancers in males and 3% of all cancers in females are attributed to alcohol exposure, especially cancer of the liver and of the digestive tract.[50] Cancer related to substance exposures at work is believed to represent between 2–20% of all cases.[51] Every year, at least 200,000 people die worldwide from cancer related to their workplaces.[52] Millions of workers run the risk of developing cancers such as lung cancer and mesothelioma from inhaling tobacco smoke or asbestos fibers on the job, or leukemia from exposure to

benzene at their workplaces.[52]

Diet and exercise

Diet, physical inactivity, and obesity are related to up to 30–35% of cancer deaths.[53] In the United States excess body weight is associated with the development of many types of cancer and is a factor in 14–20% of all cancer deaths.[53] Correspondingly, a UK study including data on over 5 million people showed higher body mass index to be related to at least 10 types of cancer, and responsible for around 12,000 cases each year in that country.[54] Physical inactivity is believed to contribute to cancer risk, not only through its effect on body weight but also through negative effects on the immune system and endocrine system.[53] More than half of the effect from diet is due to over nutrition (eating too much), rather than from eating too few vegetables or other healthful foods.

Some specific foods are linked to specific cancers. A high-salt diet is linked to gastric cancer.[55]Aflatoxin B1, a frequent food contaminate, causes liver cancer.[55]Betel nut chewing causes oral cancer.[55] The differences in dietary practices may partly explain differences in cancer incidence in different countries. For example, gastric cancer is more common in Japan due to its high-salt diet[56] and colon cancer is more common in the United States. Immigrants develop the risk of their new country, often within one generation, suggesting a substantial link between diet and cancer.[57]

Infection

Worldwide approximately 18% of cancer deaths are related to infectious diseases. This proportion varies in different regions of the world from a high of 25% in Africa to less than 10% in the developed world. Viruses are the usual infectious agents that cause cancer but cancer bacteria and parasites may also have an effect.

A virus that can cause cancer is called an *oncovirus*. These include human papillomavirus (cervical carcinoma), Epstein–Barr virus (B-cell lympho proliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B andhepatitis C viruses (hepatocellular carcinoma), and human T-cell leukemia virus-1 (T-cell leukemias). Bacterial infection may also increase the risk of cancer, as seen in *Helicobacter pylori*-induced gastric carcinoma.[58] Parasitic infections strongly associated with cancer include *Schistosoma haematobium* (squamous cell carcinoma of the bladder) and the liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis* (cholangiocarcinoma).[59]

Radiation

Up to 10% of invasive cancers are related to radiation exposure, including both ionizing radiation and non-ionizing ultraviolet radiation. Additionally, the vast majority of non-invasive cancers are non- melanoma skin cancers caused by non-ionizing ultraviolet radiation, mostly from sunlight. Sources of ionizing radiation include medical imaging and radon gas.

Ionizing radiation is not a particularly strong mutagen.[60] Residential exposure to radon gas, for example, has similar cancer risks as passive smoking.[60] Radiation is a more potent source of cancer when it is combined with other cancer-causing agents, such as radon gas exposure plus smoking tobacco.[60] Radiation can cause cancer in most parts of the body, in all animals, and at any age. Children and adolescents are twice as likely to develop radiation-induced leukemia as adults; radiation exposure before birth has ten times the effect.[60]

Medical use of ionizing radiation is a small but growing source of radiation-induced cancers. Ionizing radiation may be used to treat other cancers, but this may, in some cases, induce a second form of cancer.[60] It is also used in some kinds of medical imaging.[61]

Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies.[62] Clear evidence establishes ultraviolet radiation, especially the non-ionizing medium wave UVB, as the cause of most non-melanoma skin cancers, which are the most common forms of cancer in the world.[62]

Non-ionizing radio frequency radiation from mobile phones, electric power transmission, and other similar sources have been described as a possible carcinogen by the World Health Organization's International Agency for Research on Cancer.[63] However, studies have not found a consistent link between cell phone radiation and cancer risk.[64]

Heredity

The vast majority of cancers are non-hereditary ("sporadic cancers"). Hereditary cancers are primarily caused by an inherited genetic defect. Less than 0.3% of the population are carriers of a geneticmutation that has a large effect on cancer risk and these cause less than 3–10% of all cancer.[65] Some of these syndromes include: certain inherited mutations in the genes *BRCA1* and *BRCA2* with a more than 75% risk of breast cancer and ovarian cancer,[65] and hereditary non polyposis colorectal cancer (HNPCC or Lynch syndrome), which is present in about 3% of people with colorectal cancer,[66] among others.

Physical agents

Some substances cause cancer primarily through their physical, rather than chemical, effects on cells.[67] A prominent example of this is prolonged exposure to asbestos, naturally occurring mineral fibers that are a major cause of mesothelioma, which is a cancer of the serous membrane, usually the serous membrane surrounding the lungs.[67] Other substances in this category, including both naturally occurring and synthetic asbestos-like fibers, such as wollastonite,

attapulgite, glass wool, and rock wool, are believed to have similar effects.[67] Non-fibrous particulate materials that cause cancer include powdered metallic cobalt and nickel, and crystalline silica (quartz, cristobalite, and tridymite).[67] Usually, physical carcinogens must get inside the body (such as through inhaling tiny pieces) and require years of exposure to develop cancer.[67]

Physical trauma resulting in cancer is relatively rare.[68] Claims that breaking bones resulted in bone cancer, for example, have never been proven.[68] Similarly, physical trauma is not accepted as a cause for cervical cancer, breast cancer, or brain cancer.[68] One accepted source is frequent, long-term applicationof hot objects to the body. It is possible that repeated burns on the same part of the body, such as thoseproduced by kanger and kairo heaters (charcoal hand warmers), may produce skin cancer, especially if carcinogenic chemicals are also present.[68] Frequently drinking scalding hot tea may produce esophageal cancer.[68] Generally, it is believed that the cancer arises, or a pre-existing cancer is encouraged, during the process of repairing the trauma, rather than the cancer being caused directly by the trauma.[68] However, repeated injuries to the same tissues might promote excessive cell proliferation, which could then increase the odds of a cancerous mutation.

It is controversial whether chronic inflammation can directly cause mutation.[68,69] It is recognized, however, that inflammation can contribute to proliferation, survival, angiogenesis and migration of cancer cells by influencing the microenvironment around tumors.[70,71] Furthermore, oncogenes are known to build up an inflammatory pro-tumorigenic microenvironment.[72]

Hormones

Some hormones play a role in the development of cancer by promoting cell proliferation.[73]Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis.[74]

Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary, and testis, and also of thyroid cancer and bone cancer.[73] For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene.[73] Similarly, men of African ancestry have significantly higher levels of testosterone than men of European ancestry, and have a correspondingly much higher level of prostate cancer.[73] Men of Asian ancestry, with the lowest levels of testosterone-activating androstanediol glucuronide, have the lowest levels of prostate cancer.[73]

Other factors are also relevant

Obese people have higher levels of some hormones associated with cancer and a higher rate of those cancers.[73] Women who take hormone replacement therapy have a higher risk of developing cancers associated with those hormones.[73] On the other hand, people who exercise far more than average have lower levels of these hormones, and lower risk of cancer.[73]Osteosarcoma may be promoted by growth hormones.[73] Some treatments and prevention approaches leverage this cause by artificially reducing hormone levels, and thus discouraging hormone-sensitive cancers.[73]

Pathophysiology

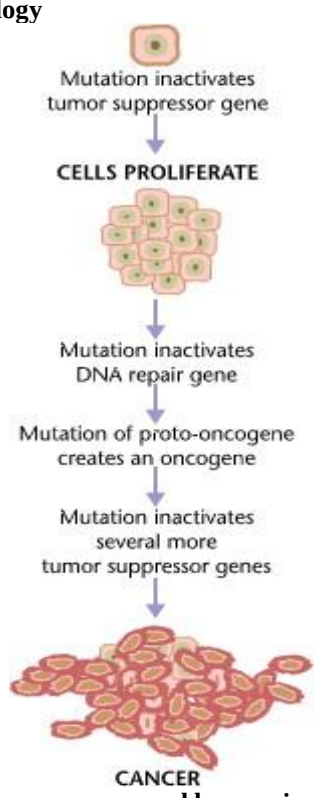


Figure-6: Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat.

Genetics

Cancer is fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes that regulate cell growth and differentiation must be altered.[75]

The affected genes are divided into two broad categories. Oncogenes are genes that promote cell growth and reproduction. Tumor suppressor genes are genes that inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over- expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in *many* genes are required to transform a normal cell into a cancer cell.[76]

Genetic changes can occur at different levels and by

different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA.

Large-scale mutations involve the deletion or gain of a portion of a chromosome. Genomic amplification occurs when a cell gains many copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well- known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in chronic myelo genous leukemia, and results in production of the BCR-ablfusion protein, an oncogenic tyrosine kinase.

Small-scale mutations include point mutations, deletions, and insertions, which may occur in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, leading to the expression of *viral* oncogenes in the affected cell and its descendants.

Replication of the enormous amount of data contained within the DNA of living cells will probabilisticallyresult in some errors (mutations). Complex error correction and prevention is built into the process, and safeguards the cell against cancer. If significant error occurs, the damaged cell can "self-destruct" through programmed cell death, termed apoptosis. If the error control processes fail, then the mutations will survive and be passed along to daughter cells.

Some environments make errors more likely to arise and propagate. Such environments can include the presence of disruptive substances called carcinogens, repeated physical injury, heat, ionising radiation, or hypoxia.[77]

The errors that cause cancer are *self-amplifying* and *compounding*, for example

- A mutation in the error-correcting machinery of a cell might cause that cell and its children to accumulate errors more rapidly.
- A further mutation in an oncogene might cause the cell to reproduce more rapidly and morefrequently than its normal counterparts.
- A further mutation may cause loss of a tumor suppressor gene, disrupting the apoptosis signalling pathway and resulting in the cell becoming immortal.
- A further mutation in signaling machinery of the cell might send error-causing signals to nearbycells.

The transformation of normal cell into cancer is akin to a chain reaction caused by initial errors, which compound into more severe errors, each progressively allowing the cell to escape the controls that limit normal tissue growth. This rebellion-like scenario becomes an undesirable survival of the fittest, where the driving forces of evolution work against the body's design and enforcement of order. Once cancer has begun to develop, this ongoing process, termed *clonal evolution*, drives progression towards more invasive stages.[78] Clonal evolution leads to intra-tumour heterogeneity that complicates designingeffective treatment strategies.

Characteristic abilities developed by cancers are divided into a number of categories. Six categories were originally proposed, in a 2000 article called "The Hallmarks of Cancer" by Douglas Hanahan and Robert Weinberg: evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, limitless replicative potential, and metastasis. Based on further work, the same authors added two more categories in 2011: reprogramming of energy metabolism and evasion of immune destruction.[38,39]

Epigenetics

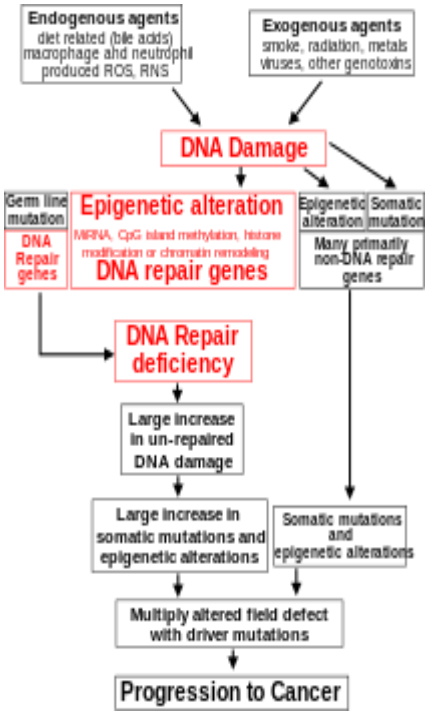


Figure-7: The central role of DNA damage and epigenetic defects in DNA repair genes in carcinogenesis.

Classically, cancer has been viewed as a set of diseases that are driven by progressive genetic abnormalities that include mutations in tumor-suppressor genes and oncogenes, and chromosomal abnormalities. However, it has become apparent that cancer is also driven by epigenetic alterations.[79]

Epigenetic alterations refer to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence. Examples of such

modifications are changes in DNA methylation (hypermethylation and hypomethylation) and histone modification[80] and changes in chromosomal architecture (caused by inappropriate expression of proteins such as HMGA2 or HMGA1).[81] Each of these epigenetic alterations serves to regulate gene expression without altering the underlying DNA sequence. These changes may remain through cell divisions, last for multiple generations, and can be considered to be epimutations (equivalent to mutations).

Epigenetic alterations occur frequently in cancers. As an example, Schnekenburger and Diederich[82] listed protein coding genes that were frequently altered in their methylation in association with colon cancer. These included 147 hyper methylated and 27 hypo methylated genes. Of the hyper methylated genes, 10 were hyper methylated in 100% of colon cancers, and many others were hyper methylated in more than 50% of colon cancers.

While large numbers of epigenetic alterations are found in cancers, the epigenetic alterations in DNA repair genes, causing reduced expression of DNA repair proteins, may be of particular importance. Such alterations are thought to occur early in progression to cancer and to be a likely cause of the genetic instability characteristic of cancers.[83,84,85,86]

Reduced expression of DNA repair genes causes deficient DNA repair. This is shown in the figure at the 4th level from the top. (In the figure, red wording indicates the central role of DNA damage and defects in DNA repair in progression to cancer.) When DNA repair is deficient DNA damages remain in cells at a higher than usual level (5th level from the top in figure), and these excess damages cause increased frequencies of mutation and/or epimutation (6th level from top of figure). Mutation rates increase substantially in cells defective in DNA mismatch repair[87,88] or in homologous re combinational repair (HRR).[89] Chromosomal rearrangements and aneuploidy also increase in HRR defective cells.[90]

Higher levels of DNA damage not only cause increased mutation (right side of figure), but also cause increased epimutation. During repair of DNA double strand breaks, or repair of other DNA damages, incompletely cleared sites of repair can cause epigenetic gene silencing.[91,92]

Deficient expression of DNA repair proteins due to an inherited mutation can cause an increased risk of cancer. Individuals with an inherited impairment in any of 34 DNA repair genes (see article DNA repair- deficiency disorder) have an increased risk of cancer, with some defects causing up to a 100% lifetime chance of cancer (e.g. p53 mutations).[93] Germ line DNA repair mutations are noted in a box on the left side of the figure, with an arrow indicating their contribution to DNA repair deficiency. However, such germline mutations (which cause highly penetrant cancer syndromes) are the cause of only about 1 percent of cancers.[94]

In sporadic cancers, deficiencies in DNA repair are occasionally caused by a mutation in a DNA repair gene, but are much more frequently caused by epigenetic alterations that reduce or silence expression of DNA repair genes. This is indicated in the figure at the 3rd level from the top. Many studies of heavy metal-induced carcinogenesis show that such heavy metals cause reduction in expression of DNA repair enzymes, some through epigenetic mechanisms. In some cases, DNA repair inhibition is proposed to be a predominant mechanism in heavy metal-induced carcinogenicity. In addition, there are frequent epigenetic alterations of the DNA sequences coding for small RNAs called microRNAs (or miRNAs). MiRNAs do not code for proteins, but can "target" protein-coding genes and reduce their expression.

Cancers usually arise from an assemblage of mutations and epi mutations that confer a selective advantage leading to clonal expansion (see Field defects in progression to cancer). Mutations, however, may not be as frequent in cancers as epigenetic alterations. An average cancer of the breast or colon canhave about 60 to 70 protein-altering mutations, of which about three or four may be "driver" mutations, and the remaining ones may be "passenger" mutations.[95]

As pointed out above under genetic alterations, cancer is caused by failure to regulate tissue growth, when the genes that regulate cell growth and differentiation are altered. It has become clear that these alterations are caused by both DNA sequence mutation in oncogenes and tumor suppressor genes as well as by epigenetic alterations. The epigenetic deficiencies in expression of DNA repair genes, in particular, likely cause an increased frequency of mutations, some of which then occur in oncogenes andtumor suppressor genes.

Metastasis

Metastasis is the spread of cancer to other locations in the body. The new tumors are called metastatic tumors, while the original is called the primary tumor. Almost all cancers can metastasize.[96] Most cancer deaths are due to cancer that has spread from its primary site to other organs (metastasized).[97]

Metastasis is very common in the late stages of cancer, and it can occur via the blood or the lymphatic system or both. The typical steps in metastasis are local invasion, intravasation into the blood or lymph, circulation through the body, extravasation into the new tissue, proliferation, and angiogenesis. Different types of cancers tend to metastasize to particular organs, but overall the most common places for metastases to occur are the lungs, liver, brain, and the bones.[96]

Diagnosis

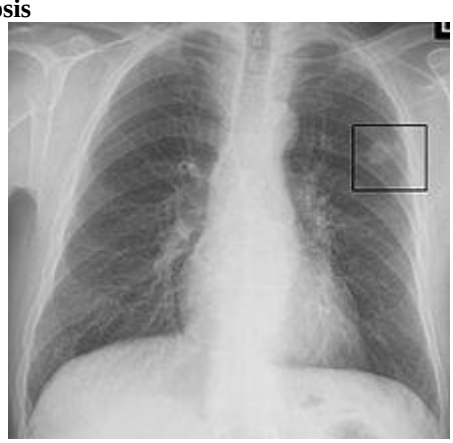


Figure-8: Chest x-ray showing lung cancer in the left lung.

Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. Neither of these lead to a definitive diagnosis, which requires the examination of a tissue sample by a pathologist. People with suspected cancer are investigated with medical tests. Thesecommonly include blood tests, X-rays, CT scans and endoscopy.

Most people are distressed to learn that they have cancer. They may become extremely anxious and depressed. The risk of suicide in people with cancer is approximately double the normal risk.[98]

Classification

Cancers are classified by the type of cell that the tumor cells resemble and is therefore presumed to be the origin of the tumor. These types include:

- Carcinoma: Cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in the aged, and include nearly all those developing in the breast, prostate, lung, pancreas, and colon.
- Sarcoma: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchymal cells outside the bone marrow.
- Lymphoma and leukemia: These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30%.[99]
- Germ cell tumor: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

Cancers are usually named using *-carcinoma*, *-sarcoma* or *-blastoma* as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called *hepatocarcinoma*, while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma, and a cancer arising from fat cells is called a *liposarcoma*. For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called *ductal carcinoma of the breast*. Here, the adjective *ductal* refers to the appearance of the cancer under the microscope, which suggests that it has originated in the milk ducts.

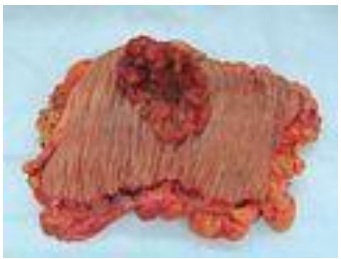
Benign tumors (which are not cancers) are named using *-oma* as a suffix with the organ name as the root. For example, a benign tumor of smooth muscle cells is called a *leiomyoma* (the common name of this frequently occurring benign tumor in the uterus is *fibroid*). Confusingly, some types of cancer use the *-noma* suffix, examples including melanoma and seminoma.

Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma, and small-cell carcinoma.

Pathology

The tissue diagnosis given by the pathologist indicates the type of cell that is proliferating, its histologicalgrade, genetic abnormalities, and other features of the tumor. Together, this information is useful to evaluate the prognosis of the patient and to choose the best treatment. Cytogenetics and immunohistochemistry are other types of testing that the pathologist may perform on the tissue specimen. These tests may provide information about the molecular changes (such as mutations, fusion genes, and numerical chromosome changes) that have happened in the cancer cells, and may thus also indicate the future behavior of the cancer (prognosis) and best treatment.

Figure-9: An invasive ductal carcinoma of the breast (pale area at the center) surrounded by spikes ofwhitish scar tissue and yellow fatty tissue



An invasive colorectal carcinoma (top center) in a colectomy specimen

A squamous-cell carcinoma (the whitish tumor) near the bronchi in a lung specimen.

A large invasive ductal carcinoma in a mastectomy specimen

Prevention

Cancer prevention is defined as active measures to decrease the risk of cancer.[100] The vast majority of cancer cases are due to environmental risk factors, and many, but not all, of these environmental factors are controllable lifestyle choices. Thus, cancer is considered a largely preventable disease.[101] Between 70% and 90% of common cancers are due to environmental factors and therefore possibly preventable.[102]

Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, overweight / obesity, an insufficient diet, physical inactivity, alcohol, sexually transmitted infections, and air pollution.[103] Not all environmental causes are controllable, such as naturally occurring background radiation, and other cases of cancer are caused through hereditary genetic disorders, and thus it is not possible to prevent all cases of cancer.

Dietary

While many dietary recommendations have been proposed to reduce the risk of cancer, the evidence to support them is not definitive.[104] The primary dietary factors that increase risk are obesity and alcohol consumption; with a diet low in fruits and vegetables and high in red meat being implicated but not confirmed.[105,106] A 2014 meta-analysis did not find a relationship between fruits and vegetables and cancer.[107] Consumption of coffee is associated with a reduced risk of liver cancer.[108] Studies have linked excessive consumption of red or processed meat to an increased risk of breast cancer, colon cancer, and pancreatic cancer, a phenomenon that could be due to the presence of carcinogens in meats cooked at high temperatures.[109,110] This was confirmed in 2015 by the IARC of the World Health Organization, which determined that eating processed meat (e.g., bacon, ham, hot dogs, sausages) and, to a lesser degree, red meat was linked to some cancers.[111,112]

Dietary recommendations for cancer prevention typically include an emphasis on vegetables, fruit, whole grains, and fish, and an avoidance of processed and red meat (beef, pork, lamb), animal fats, and refined carbohydrates.[104]

Medication

The concept that medications can be used to prevent cancer is attractive, and evidence supports their use in a few defined circumstances.[113] In the general population, NSAIDs reduce the risk of colorectal cancer, however due to the cardiovascular and gastrointestinal side effects they cause overall harm when used for prevention.[114]Aspirin has been found to reduce the risk of death from cancer by about 7%.[115]COX-2 inhibitor may decrease the rate of polyp formation in people with familial adenomatous polyposis, however it is associated with the same adverse effects as NSAIDs.[116] Daily use of tamoxifen or raloxifene has been demonstrated to reduce the risk of developing breast cancer in high-risk women.[117] The benefit versus harm for 5-alpha-reductase inhibitor such as finasteride is not clear.[118]

Vitamins have not been found to be effective at preventing cancer,[119] although low blood levels of vitamin D are correlated with increased cancer risk.[120,121] Whether this relationship is causal and vitamin D supplementation is protective is not determined.[122]Beta-Carotene supplementation has been found to increase lung cancer rates in those who are high risk.[123]Folic acid supplementation has not been found effective in preventing colon cancer and may increase colon polyps.[124] It is unclear if selenium supplementation has an effect.[125]

Vaccination

Vaccines have been developed that prevent infection by some carcinogenic viruses.[112,126]Human papillomavirus vaccine (Gardasil and Cervarix) decreases the risk of developing cervical cancer.[112,126] The hepatitis B vaccine prevents infection with hepatitis B virus and thus decreases the risk of liver cancer.[126] The administration of human papillomavirus and hepatitis B vaccinations is recommended when resources allow.[127]

Screening

Unlike diagnosis efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear.[128] This may involve physical examination, blood or urine tests, or medical imaging.[128]

Cancer screening is currently not possible for many types of cancers, and even when tests are available, they may not be recommended for everyone. *Universal screening* or *mass screening* involves screening everyone.[129] *Selective screening* identifies people who are known to be at higher risk of developing cancer, such as people with a family history of cancer.[129] Several factors are considered to determine whether the benefits of screening outweigh the risks and the costs of screening.[128] These factorsinclude:

- Possible harms from the screening test: for example, X-ray images involve exposure to potentially harmful ionizing radiation.
- The likelihood of the test correctly identifying cancer.
- The likelihood of cancer being present: Screening is not normally useful for rare cancers.
- Possible harms from follow-up procedures.
- Whether suitable treatment is available.
- Whether early detection improves treatment outcomes.
- Whether the cancer will ever need treatment.
- Whether the test is acceptable to the people: If a screening test is too burdensome (forexample, being extremely painful), then people will refuse to participate.
- Cost of the test.

Recommendations

The U.S. Preventive Services Task Force (USPSTF) strongly recommends cervical cancer screening in women who are sexually active and have a cervix at least until the age of 65.[130] They recommend that Americans be screened for colorectal cancer via fecal occult blood testing, sigmoidoscopy, or colonoscopy starting at age 50 until age 75.[131] There is insufficient evidence to recommend for or against screening for skin cancer,[132]oral cancer,[133] lung cancer,[134] or prostate cancer in men under 75.[135] Routine screening is not recommended for bladder cancer,[136] testicular cancer,[137] ovarian cancer,[138] pancreatic cancer,[139] or prostate cancer.[140]

The USPSTF recommends mammography for breast cancer screening every two years for those 50– 74 years old; however, they do not recommend either breast self-examination or clinical breast examination.[141] A 2011 Cochrane review came to slightly different conclusions with respect to breast cancer screening stating that routine mammography may do more harm than good.[142]

Japan screens for gastric cancer using photofluorography due to the high incidence there.[33]

Genetic testing

Genetic testing for individuals at high-risk of certain cancers is recommended.[127][143] Carriers of these mutations may then undergo enhanced surveillance, chemoprevention, or preventative surgery to reduce their subsequent risk.[143]

Management

Many treatment options for cancer exist, with the primary ones including surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care. Which treatments are used depends on the type, location, and grade of the cancer as well as the person's health and wishes. The treatment intent may be curative or not curative.

Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. The term encompasses any of a large variety of different anticancer drugs, which are divided into broad categories such as alkylating agents and antimetabolites.[144] Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells.

Targeted therapy is a form of chemotherapy that targets specific molecular differences between cancer and normal cells. The first targeted therapies to be developed blocked the estrogen receptor molecule, inhibiting the growth of breast cancer. Another common example is the class of Bcr-Abl inhibitors, which are used to treat chronic myelogenous leukemia (CML).[145] Currently, there are targeted therapies for breast cancer, multiple myeloma, lymphoma, prostate cancer, melanoma and other cancers.[146]

The efficacy of chemotherapy depends on the type of cancer and the stage. In combination with surgery, chemotherapy has proven useful in a number of different cancer types including: breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer, and certain lung cancers.[147] The overall effectiveness ranges from being curative for some cancers, such as some leukemias,[148][149] to being ineffective, such as in some brain tumors,[150] to being needless in others, like most non-melanoma skin cancers.[151] The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Even when it is impossible for chemotherapy to provide a permanent cure, chemotherapy may be useful to reduce symptoms like pain or to reduce the size of an inoperable tumor in the hope that surgery will be possible in the future.

Radiation

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer. It works by damaging the DNA of cancerous tissue leading to cellular death. To spare normal tissues (such as skin or organs, which radiation must pass through to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose there than

in the surrounding, healthy tissue. As with chemotherapy, different cancers respond differently to radiation therapy.[152,153,154]

Radiation therapy is used in about half of all cases and the radiation can be from either internal sources in the form of brachytherapy or external radiation sources. The radiation is most commonly low energy x-rays for treating skin cancers while higher energy x-ray beams are used in the treatment of cancers within the body.[155] Radiation is typically used in addition to surgery and or chemotherapy but for certain types of cancer, such as early head and neck cancer, may be used alone.[156] For painful bone metastasis, it has been found to be effective in about 70% of people.[156]

Surgery

Surgery is the primary method of treatment of most isolated solid cancers and may play a role in palliation and prolongation of survival. It is typically an important part of making the definitive diagnosis and staging the tumor as biopsies are usually required. In localized cancer surgery typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area. For some types of cancer this is all that is needed to eliminate the cancer.[147]

Palliative care

Palliative care refers to treatment that attempts to make the person feel better and may or may not be combined with an attempt to treat the cancer. Palliative care includes action to reduce the physical, emotional, spiritual, and psycho-social distress experienced by people with cancer. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to improve the person's quality of life.

People at all stages of cancer treatment should have some kind of palliative care to provide comfort. In some cases, medical specialtyprofessional organizations recommend that people and physicians respond to cancer only with palliative care and not with cure-directed therapy.[157] This includes:[158]

1. People with low performance status, corresponding with limited ability to care forthemselves[157]
2. People who received no benefit from prior evidence-based treatments[157]
3. People who are not eligible to participate in any appropriate clinical trial[157]
4. People for whom the physician sees no strong evidence that treatment would be effective[157]

Palliative care is often confused with hospice and therefore only involved when people approach end of life. Like hospice care, palliative care attempts to help the person cope with the immediate needs and toincrease the person's comfort. Unlike hospice care, palliative care does not require people to stop treatment aimed at prolonging their lives or curing the cancer.

Multiple national medical guidelines recommend early palliative care for people whose cancer has produced distressing symptoms (pain, shortness of breath, fatigue, nausea) or who need help coping with their illness. In people who have metastatic disease when first diagnosed, oncologists should consider a palliative care consult immediately. Additionally, an oncologist should consider a palliative care consult in any person they feel has less than 12 months of life even if continuing aggressive treatment.[159,160,161]

Immunotherapy

A variety of therapies using immunotherapy, stimulating or helping the immune system to fight cancer,have come into use since 1997, and this continues to be an area of very active research.[162]

Alternative medicine

Complementary and alternative cancer treatments are a diverse group of health care systems, practices, and products that are not part of conventional medicine.[163] "Complementary medicine" refers to methods and substances used along with conventional medicine, while "alternative medicine" refers to compounds used instead of conventional medicine.[164] Most complementary and alternative medicines for cancer have not been rigorously studied or tested. Some alternative treatments have been investigated and shown to be ineffective but still continue to be marketed and promoted. Cancer researcher Andrew J. Vickers has stated: "The label 'unproven' is inappropriate for such therapies; it is time to assert that many alternative cancer therapies have been 'disproven'."[165]

Prognosis

Cancer has a reputation as a deadly disease. Taken as a whole, about half of people receiving treatment for invasive cancer (excluding carcinoma in situ and non-melanoma skin cancers) die from cancer or its treatment.[33] Survival is worse in the developing world,[33] partly because the types of cancer that are most common there are at present harder to treat than those associated with the lifestyle of developed countries.[166] However, the survival rates vary dramatically by type of cancer, and by the stage at which it is diagnosed, with the range running from the great majority of people surviving to almost no one surviving as long as five years after diagnosis. Once a cancer has metastasized or spread beyond its original site, the prognosis normally becomes much worse.

Those who survive cancer are at increased risk of developing a second primary cancer at about twice the rate of those never diagnosed with cancer.[167] The increased risk is believed to be primarily due to the same risk factors that produced the first cancer, partly due to the treatment for the first cancer, and potentially related to better compliance with screening.[167]

Predicting either short-term or long-term survival is

difficult and depends on many factors. The most important factors are the particular kind of cancer and the patient's age and overall health. People who are frail with many other health problems have lower survival rates than otherwise healthy people. A centenarian is unlikely to survive for five years even if the treatment is successful. People who report a higher quality of life tend to survive longer.[168] People with lower quality of life may be affected by major depressive disorder and other complications from cancer treatment and/or disease progression that both impairs their quality of life and reduces their quantity of life. Additionally, patients with worse prognoses may be depressed or report a lower quality of life directly because they correctly perceive that their condition is likely to be fatal.

People with cancer, even those who are walking on their own, have an increased risk of blood clots in veins. The use of heparin appears improve survival and decrease the risk of blood clots.[169]

Epidemiology

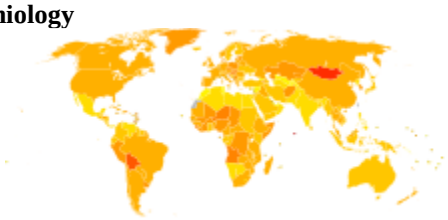


Figure-10: Death rate adjusted for age for malignant cancer per 100,000 inhabitants in 2004[170]

no data ≤ 55	180–205
55–80	205–230
80–105	230–255
105–130	255–280
130–155	280–305
155–180	≥ 305

In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers),[33] and in 2010 nearly 7.98 million people died.[171] Cancers as a group account for approximately 13% of all deaths each year with the most common being: lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths).[172] This makes invasive cancer the leading causeof death in the developed world and the second leading cause of death in the developing world.[33] Over half of cases occur in the developing world.[33]

Deaths from cancer were 5.8 million in 1990[171] and rates have been increasing primarily due to an aging population and lifestyle changes in the developing world.[33] The most significant risk factor for developing cancer is old age.[173] Although it is possible for cancer to strike at any age, most people who are diagnosed with invasive cancer are over the age of 65.[173] According to cancer researcher Robert A. Weinberg, "If we lived long enough, sooner or later we all would get cancer."[174] Some of the association between aging and cancer is attributed to immunosenescence,[175] errors accumulated in DNA over a lifetime,[176] and age-related changes in the endocrine system.[177] The effect of aging on cancer is complicated with a number of factors such as DNA damage and inflammation promoting it and a number of factors such as vascular aging and endocrine changes inhibiting it.[178]

Some slow-growing cancers are particularly common. Autopsy studies in Europe and Asia have shown that up to 36% of people have undiagnosed and apparently harmless thyroid cancer at the time of their deaths, and that 80% of men develop prostate cancer by age 80.[179,180] As these cancers did not cause the person's death, identifying them would have represented overdiagnosis rather than useful medical care.

The three most common childhood cancers are leukemia (34%), brain tumors (23%), and lymphomas (12%).[181] In the United States cancer affects about 1 in 285 children.[182] Rates of childhood cancer have increased by 0.6% per year between 1975 to 2002 in the United States[183] and by 1.1% per year between 1978 and 1997 in Europe.[181] Death from childhood cancer have decreased by half since 1975in the United States.[182]

History

Figure-11: Engraving with two views of a Dutch woman who had a tumor removed from her neck in 1689.

Cancer has existed for all of human history.[184] The earliest written record regarding cancer is from circa 1600 BC in the Egyptian Edwin Smith Papyrus and describes cancer of the breast.[184] Hippocrates (ca. 460 BC – ca. 370 BC) described several kinds of cancer, referring to them with the Greek word καρκίνος*karkinos* (crab or crayfish).[184] This name comes from the appearance of the cut surface of a solid malignant tumor, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name".[185] Galen stated that "cancer of the breast is so called because of the fancied resemblance to a crab given by the lateral prolongations of the tumor and the adjacent distended veins",[738] Celsus (ca. 25 BC – 50 AD) translated *karkinos* into the Latin*cancer*, also meaning crab and recommended surgery as treatment.[184]Galen (2nd century AD) disagreed with the use of surgery and recommended purgatives instead.[184] These

recommendations largely stood for 1000 years.[184]

In the 15th, 16th and 17th centuries, it became acceptable for doctors to dissect bodies to discover the cause of death.[187] The German professor Wilhelm Fabry believed that breast cancer was caused by a milk clot in a mammary duct. The Dutch professor Francois de la Boe Sylvius, a follower of Descartes, believed that all disease was the outcome of chemical processes, and that acidic lymph fluid was the cause of cancer. His contemporary Nicolaes Tulp believed that cancer was a poison that slowly spreads, and concluded that it was contagious.[188]

The physician John Hill described tobacco snuff as the cause of nose cancer in 1761.[187] This was followed by the report in 1775 by British surgeon Percivall Pott that chimney sweeps' carcinoma, a cancer of the scrotum, was a common disease among chimney sweeps.[189] With the widespread use of the microscope in the 18th century, it was discovered that the 'cancer poison' spread from the primary tumor through the lymph nodes to other sites ("metastasis"). This view of the disease was first formulated by the English surgeon Campbell De Morgan between 1871 and 1874.[190]

Society and culture

Though many diseases (such as heart failure) may have a worse prognosis than most cases of cancer, cancer is the subject of widespread fear and taboos. The euphemism "after a long illness" is still commonly used (2012), reflecting an apparent stigma.[191] This deep belief that cancer is necessarily a difficult and usually deadly disease is reflected in the systems chosen by society to compile cancer statistics: the most common form of cancer non-melanoma skin cancers, accounting for about one- third of all cancer cases worldwide, but very few deaths[192,193] are excluded from cancer statistics specifically because they are easily treated and almost always cured, often in a single, short, outpatient procedure.[194]

Cancer is regarded as a disease that must be "fought" to end the "civil insurrection"; a War on Cancer has been declared. Military metaphors are particularly common in descriptions of cancer's human effects, and they emphasize both the parlous state of the affected individual's health and the need for the individual to take immediate, decisive actions himself, rather than to delay, to ignore, or to rely entirely on others caring for him. The military metaphors also help rationalize radical, destructivetreatments.[195,196]

In the 1970s, a relatively popular alternative cancer treatment was a specialized form of talk therapy, based on the idea that cancer was caused by a bad attitude.[197] People with a "cancer personality"— depressed, repressed, self-loathing, and afraid to express their emotions—were believed to have manifested cancer through subconscious desire. Some psychotherapists said that treatment to change the patient's outlook on life would cure the cancer.[197] Among other effects, this belief allows society to blame the victim for having caused the cancer (by "wanting" it) or having prevented its cure (by not becoming a sufficiently happy, fearless, and loving person).[198] It also increases patients' anxiety, as they incorrectly believe that natural emotions of sadness, anger or fear shorten their lives.[198] The idea was excoriated by the notoriously outspoken Susan Sontag, who published *Illness as Metaphor* while recovering from treatment for breast cancer in 1978.[197] Although the original idea is now generally regarded as nonsense, the idea partly persists in a reduced form with a widespread, but incorrect, belief that deliberately cultivating a habit of positive thinking will increase survival.[198] This notion is particularly strong in breast cancer culture.[198]

One idea about why people with cancer are blamed or stigmatized, called the just-world hypothesis, is that blaming cancer on the patient's actions or attitudes allows the blamers to regain a sense of control. This is based upon the blamers' belief that the world is fundamentally just, and so any dangerous illness, like cancer, must be a type of punishment for bad choices, because in a just world, bad things would not happen to good people.[199]

Economic effect

In 2007, the overall costs of cancer in the U.S. — including treatment and indirect mortality expenses (such as lost productivity in the workplace) — was estimated to be $226.8 billion. In 2009, 32% of Hispanics and 10% of children 17 years old or younger lacked health insurance; "uninsured patients and those from ethnic minorities are substantially more likely to be diagnosed with cancer at a later stage, when treatment can be more extensive and more costly."[200]

Research

Figure-12: University of Florida Cancer Hospital.

Because cancer is a class of diseases,[201,202] it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases.[189,203]Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but this has not been the case in practice.[,204] It is more likely that angiogenesis inhibitors and other cancer therapeutics will

be used in combination to reduce cancer morbidity and mortality.[205]

Experimental cancer treatments are treatments that are being studied to see whether they work. Typically, these are studied in clinical trials to compare the proposed treatment to the best existing treatment. They may be entirely new treatments, or they may be treatments that have been used successfully in one type of cancer, and are now being tested to see whether they are effective in another type.[206] More and more, such treatments are being developed alongside companion diagnostic tests to target the right drugs to the right patients, based on their individual biology.[207]

Cancer research is the intense scientific effort to understand disease processes and discover possible therapies.
Research about cancer causes focuses on the following issues

- Agents (e.g. viruses) and events (e.g. mutations) that cause or facilitate genetic changes in cells destined to become cancer.
- The precise nature of the genetic damage, and the genes that are affected by it.
- The consequences of those genetic changes on the biology of the cell, both in generating the defining properties of a cancer cell, and in facilitating additional genetic events that lead to further progression of the cancer.

The improved understanding of molecular biology and cellular biology due to cancer research has led to a number of new treatments for cancer since U.S. President Nixon declared the "War on Cancer" in 1971. Since then, the U.S. has spent over $200 billion on cancer research, including resources from the public and private sectors and foundations.[208] During that time, the country has seen a five percent decrease in the cancer death rate (adjusting for size and age of the population) between 1950 and 2005.[209]

Hypercompetition for the financial resources that are required to conduct science appears to suppress the creativity, cooperation, risk-taking, and original thinking required to make fundamental discoveries, unduly favoring low-risk research into small incremental advancements over innovative research that might discover radically new and dramatically improved therapy. Other consequences of the highly pressured competition for research resources appear to be a substantial number of research publications whose results cannot be replicated, and perverse incentives in research funding that encourage grantee institutions to grow without making sufficient investments in their own faculty and facilities.[210,211,212,213]

Pregnancy
Because cancer is largely a disease of older adults, it is not common in pregnant women. Cancer affects approximately 1 in 1,000 pregnant women.[214] The most common cancers found during pregnancy are the same as the most common cancers found in non-pregnant women during childbearing ages: breast cancer, cervical cancer, leukemia, lymphoma, melanoma, ovarian cancer, and colorectal cancer.[214]

Diagnosing a new cancer in a pregnant woman is difficult, in part because any symptoms are commonly assumed to be a normal discomfort associated with pregnancy.[214] As a result, cancer is typically discovered at a somewhat later stage than average in many pregnant or recently pregnant women. Some imaging procedures, such as MRIs (magnetic resonance imaging), CT scans, ultrasounds, and mammograms with fetal shielding are considered safe during pregnancy; some others, such as PET scansare not.[214]

Treatment is generally the same as for non-pregnant women.[214] However, radiation and radioactive drugs are normally avoided during pregnancy, especially if the fetal dose might exceed 100 cGy. In some cases, some or all treatments are postponed until after birth if the cancer is diagnosed late in the pregnancy. Early deliveries to speed the start of treatment are not uncommon. Surgery is generally safe, but pelvic surgeries during the first trimester may cause miscarriage. Some treatments, especially certain chemotherapy drugs given during the first trimester, increase the risk of birth defects and pregnancy loss (spontaneous abortions and stillbirths).[214]

Elective abortions are not required and, for the most common forms and stages of cancer, do not improve the likelihood of the mother surviving or being cured.[214] In a few instances, such as advanced uterine cancer, the pregnancy cannot be continued, and in others, such as an acute leukemia discovered early in pregnancy, the pregnant woman may choose to have an abortion so that she can begin aggressive chemotherapy without worrying about birth defects.[214]

Some treatments may interfere with the mother's ability to give birth vaginally or to breastfeed her baby.[214] Cervical cancer may require birth by Caesarean section. Radiation to the breast reduces the ability of that breast to produce milk and increases the risk of mastitis. Also, when chemotherapy is being given after birth, many of the drugs pass through breast milk to the baby, which could harm the baby.[214]

Other animals
Veterinary oncology, concentrating mainly on cats and dogs, is a growing specialty in wealthy countries, and the major forms of human treatment such as surgery and radiotherapy may be offered. The most common types of cancer differ, but the cancer burden seems at least as high in pets as in humans. Animals, typically rodents, are often used in cancer research, and studies of natural cancers in larger animals may benefit research into

human cancer.[215]

In non-humans, a few types of transmissible cancer have been described, wherein the cancer spreads between animals by transmission of the tumor cells themselves. This phenomenon is seen in dogs with Sticker's sarcoma, also known as canine transmissible venereal tumor, as well as devil facial tumor disease in Tasmanian devils.

Types of Cancers

Cancer is a group of diseases that involve abnormal increases in the number of cells, with the potential to invade or spread to other parts of the body.[217] Not all tumors or lumps are cancerous; benign tumors are not classified as being cancer because they do not spread to other parts of the body.[217] There are over 100 different known cancers that affect humans.[217]

Cancers are often described by the body part that they originated in. However, some body parts contain multiple types of tissue, so for greater precision, cancers are additionally classified by the type of cell that the tumor cells originated from. These types include

- *Carcinoma*: Cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in older adults. Nearly all cancers developing in the breast, prostate, lung, pancreas, and colon are carcinomas.
- *Sarcoma*: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develop from cells originating in mesenchymal cells outside the bone marrow.
- *Lymphoma* and *leukemia*: These two classes of cancer arise from cells that make blood. Leukemia is the most common type of cancer in children accounting for about 30%.[218] However, far more adults develop lymphoma and leukemia.
- *Germ cell tumor*: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- *Blastoma*: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

Cancers are usually named using *-carcinoma*, *-sarcoma* or *-blastoma* as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called *hepatocarcinoma*, while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma, and a cancer arising from fat cells is called a *liposarcoma*. For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called *ductal carcinoma of the breast*. Here, the adjective *ductal* refers to the appearance of the cancer under the microscope, which suggests that it has originated in the milk ducts.

Benign tumors (which are not cancers) are usually named using *-oma* as a suffix with the organ name as the root. For example, a benign tumor of smooth muscle cells is called a *leiomyoma* (the common name of this frequently occurring benign tumor in the uterus is *fibroid*). Confusingly, some types of cancer use the *-noma* suffix, examples including melanoma and seminoma.

Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma, and small-cell carcinoma.

Types:[205,219]

A

- Acute lymphoblastic leukemia (ALL)
- Acute myeloid leukemia
- Adrenocortical carcinoma
- AIDS-related cancers
- AIDS-related lymphoma
- Anal cancer
- Appendix cancer
- Astrocytoma, childhood cerebellar or cerebral

B

- Basal-cell carcinoma
- Bile duct cancer, extrahepatic (see cholangiocarcinoma)
- Bladder cancer
- Bone tumor, osteosarcoma/malignant fibrous histiocytoma
- Brainstem glioma
- Brain cancer
- Brain tumor, cerebellar astrocytoma
- Brain tumor, cerebral astrocytoma/malignant glioma
- Brain tumor, ependymoma
- Brain tumor, medulloblastoma
- Brain tumor, supratentorial primitive neuroectodermal tumors
- Brain tumor, visual pathway and hypothalamic glioma
- Breast cancer
- Bronchial adenomas/carcinoids
- Burkitt's lymphoma

C

- Carcinoid tumor, childhood
- Carcinoid tumor, gastrointestinal
- Carcinoma of unknown primary
- Central nervous system lymphoma, primary
- Cerebellar astrocytoma, childhood
- Cerebral astrocytoma/malignant glioma, childhood
- Cervical cancer
- Childhood cancers
- Chondrosarcoma
- Chronic lymphocytic leukemia
- Chronic myelogenous leukemia
- Chronic myeloproliferative disorders
- Colon cancer

- Cutaneous T-cell lymphoma

D
- Desmoplastic small round cell tumor

E
- Endometrial cancer
- Ependymoma
- Epitheliod Hemangioendothelioma (EHE)
- Esophageal cancer
- Ewing's sarcoma in the Ewing family of tumors
- Extracranial germ cell tumor, childhood
- Extragonadal germ cell tumor
- Extrahepatic bile duct cancer
- Eye cancer, intraocular melanoma
- Eye cancer, retinoblastoma

G
- Gallbladder cancer
- Gastric (stomach) cancer
- Gastrointestinal carcinoid tumor
- Gastrointestinal stromal tumor (GIST)
- Germ cell tumor: extracranial, extragonadal, or ovarian
- Gestational trophoblastic tumor
- Glioma of the brain stem
- Glioma, childhood cerebral astrocytoma
- Glioma, childhood visual pathway and hypothalamic
- Gastric carcinoid

H
- Hairy cell leukemia
- Head and neck cancer
- Heart cancer
- Hepatocellular (liver) cancer
- Hodgkin lymphoma
- Hypopharyngeal cancer
- Hypothalamic and visual pathway glioma, childhood

I
- Intraocular melanoma
- Islet cell carcinoma (endocrine pancreas)

K
- Kaposi sarcoma
- Kidney cancer (renal cell cancer)

L
- Laryngeal cancer
- Leukaemias
- Leukaemia, acute lymphoblastic (also called acute lymphocytic leukaemia)
- Leukaemia, acute myeloid (also called acute myelogenous leukemia)
- Leukaemia, chronic lymphocytic (also called chronic lymphocytic leukemia)
- Leukemia, chronic myelogenous (also called chronic myeloid leukemia)
- Leukemia, hairy cell
- Lip and oral cavity cancer
- Liposarcoma
- Liver cancer (primary)
- Lung cancer, non-small cell
- Lung cancer, small cell
- Lymphomas
- Lymphoma, AIDS-related
- Lymphoma, Burkitt
- Lymphoma, cutaneous T-Cell
- Lymphoma, Hodgkin
- Lymphomas, Non-Hodgkin (an old classification of all lymphomas except Hodgkin's)
- Lymphoma, primary central nervous system

M
- Macroglobulinemia, Waldenström
- Male breast cancer
- Malignant fibrous histiocytoma of bone/osteosarcoma
- Medulloblastoma, childhood
- Melanoma
- Melanoma, intraocular (eye)
- Merkel cell cancer
- Mesothelioma, adult malignant
- Mesothelioma, childhood
- Metastatic squamous neck cancer with occult primary
- Mouth cancer
- Multiple endocrine neoplasia syndrome, childhood
- Multiple myeloma/plasma cell neoplasm
- Mycosis fungoides
- Myelodysplastic syndromes
- Myelodysplastic/myeloproliferative diseases
- Myelogenous leukemia, chronic
- Myeloid leukemia, adult acute
- Myeloid leukemia, childhood acute
- Myeloma, multiple (cancer of the bone-marrow)
- Myeloproliferative disorders, chronic
- Myxoma

N
- Nasal cavity and paranasal sinus cancer
- Nasopharyngeal carcinoma
- Neuroblastoma
- Non-Hodgkin lymphoma
- Non-small cell lung cancer

O
- Oligodendroglioma
- Oral cancer
- Oropharyngeal cancer
- Osteosarcoma/malignant fibrous histiocytoma of bone
- Ovarian cancer
- Ovarian epithelial cancer (surface epithelial-stromal tumor)

- Ovarian germ cell tumor
- Ovarian low malignant potential tumor

P

- Pancreatic cancer
- Pancreatic cancer, islet cell
- Paranasal sinus and nasal cavity cancer
- Parathyroid cancer
- Penile cancer
- Pharyngeal cancer
- Pheochromocytoma
- Pineal astrocytoma
- Pineal germinoma
- Pineoblastoma and supratentorial primitive neuroectodermal tumors, childhood
- Pituitary adenoma
- Plasma cell neoplasia/Multiple myeloma
- Pleuropulmonary blastoma
- Primary central nervous system lymphoma
- Prostate cancer

R

- Rectal cancer
- Renal cell carcinoma (kidney cancer)
- Renal pelvis and ureter, transitional cell cancer
- Retinoblastoma
- Rhabdomyosarcoma, childhood

S

- Salivary gland cancer
- Sarcoma, Ewing family of tumors
- Sarcoma, Kaposi
- Sarcoma, soft tissue
- Sarcoma, uterine
- Sézary syndrome
- Skin cancer (non-melanoma)
- Skin cancer (melanoma)
- Skin carcinoma, Merkel cell
- Small cell lung cancer
- Small intestine cancer
- Soft tissue sarcoma
- Squamous cell carcinoma – see skin cancer (non-melanoma)
- Squamous neck cancer with occult primary, metastatic
- Stomach cancer
- Supratentorial primitive neuroectodermal tumor, childhood

T

- T-Cell lymphoma, cutaneous – see Mycosis Fungoides and Sézary syndrome
- Testicular cancer
- Throat cancer
- Thymoma, childhood
- Thymoma and thymic carcinoma
- Thyroid cancer
- Thyroid cancer, childhood
- Transitional cell cancer of the renal pelvis and ureter
- Trophoblastic tumor, gestational

U

- Unknown primary site, carcinoma of, adult
- Unknown primary site, cancer of, childhood
- Ureter and renal pelvis, transitional cell cancer
- Urethral cancer
- Uterine cancer, endometrial
- Uterine sarcoma

V

- Vaginal cancer
- Visual pathway and hypothalamic glioma, childhood
- Vulvar cancer

W

- Waldenström macroglobulinemia
- Wilms tumor (kidney cancer),childhood

Cell Line

The development and various other aspects of primary culture are described. The term cell line refers to the propagation of culture after the first subculture.

In other words, once the primary culture is sub-cultured, it becomes a cell line. A given cell line contains several cell lineages of either similar or distinct phenotypes.

It is possible to select a particular cell lineage by cloning or physical cell separation or some other selection method. Such a cell line derived by selection or cloning is referred to as cell strain. Cell strains do not have infinite life, as they die after some divisions.

Types of Cell Lines

Finite Cell Lines

The cells in culture divide only a limited number of times, before their growth rate declines and they eventually die. The cell lines with limited culture life spans are referred to as finite cell lines. The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction. The actual number of doublings depends on the species, cell lineage differences, culture conditions etc. The human cells generally divide 50-100 times, while murine cells divide 30-50 times before dying.

Continuous Cell Lines

A few cells in culture may acquire a different morphology and get altered. Such cells are capable of growing faster resulting in an independent culture. The progeny derived from these altered cells has unlimited life (unlike the cell strains from which they originated). They are designated as continuous cell lines.

The continuous cell lines are transformed, immortal and tumorigenic. The transformed cells for continuous cell lines may be obtained from normal primary cell cultures (or cells strains) by treating them with chemical carcinogens or by infecting with oncogenic viruses. In the Table. 36.1, the different properties of finite cell lines and continuous cell lines are compared.

Table-1: The most commonly used terms while dealing with cell lines are explained below.

TABLE 36.1 Comparison of properties of finite and continuous cell lines

Property	*Finite cell line*	*Continuous cell line*
Growth rate	Slow	Fast
Mode of growth	Monolayer	Suspension or monolayer
Yield	Low	High
Transformation	Normal	Immortal, tumorigenic
Ploidy	Euploid (multiple of haploid chromosomes)	Aneuploid (not an exact multiple of haploid chromosomes)
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Cloning efficiency	Low	High
Serum requirement	High	Low
Markers	Tissue specific	Chromosomal, antigenic or enzymatic

Split ratio

The divisor of the dilution ratio of a cell culture at subculture. For instance, when each subculture divided the culture to half, the split ratio is 1: 2.

Passage number

It is the number of times that the culture has been sub-cultured.

Generation number

It refers to the number of doublings that a cell population has undergone. It must be noted that the passage number and generation number are not the same, and they are totally different.

Nomenclature of Cell Lines

It is a common practice to give codes or designations to cell lines for their identification. For instance, the code NHB 2-1 represents the cell line from normal human brain, followed by cell strain (or cell line number) 2 and clone number 1. The usual practice in a culture laboratory is to maintain a log book or computer database file for each of the cell lines.

While naming the cell lines, it is absolutely necessary to ensure that each cell line designation is unique so that there occurs no confusion when reports are given in literature. Further, at the time of publication, the-cell line should be prefixed with a code designating the laboratory from which it was obtained e.g. NCI for National Cancer Institute, Wl for Wistar Institute.

Commonly used cell lines

There are thousands of cell lines developed from different laboratories world over. A selected list of some commonly used cell lines along with their origin, morphology and other characters are given inTable-2.

Table-2:

TABLE 36.2 A selected list of commonly used cell lines

Cell line	*Species of origin*	*Tissue of origin*	*Morphology*	*Ploidy*	*Characteristics*
IMR-90	Human	Lung	Fibroblast	Diploid	Susceptible to human viral infections.
3T3-A31	Mouse	Connective tissue	Fibroblast	Aneuploid	Contact inhibited, readily transformed
BHK21-C13	Hamster (Syrian)	Kidney	Fibroblast	Aneuploid	Readily transformable
CHO-k1	Chinese hamster	Ovary	Fibroblast	Diploid	Simple karyotype
NRK49F	Rat	Kidney	Fibroblast	Aneuploid	Induction of suspension growth by TGF-α, β.
BRL 3A	Rat	Liver	Epithelial	Diploid	Produces IGF-2
Vero	Monkey	Kidney	Fibroblast	Aneuploid	Viral substrate and assay
HeLa-S_3	Human	Cervical carcinoma	Epithelial	Aneuploid	Rapid growth, high plating efficiency.
Sk/HEP-I	Human	Hepatoma	Endothelial	Aneuploid	Factor VIII
Caco-2	Human	Colo-rectal carcinoma	Epithelial	Aneuploid with polarised support.	Forms tight monolayer
MCF-7	Human	Breast tumor (effusion)	Epithelial	Aneuploid	Estrogen receptor positive.
Friend	Mouse	Spleen	Suspension	Aneuploid	Hemoglobin, growth hormone.

Colon Cancer
Colorectal cancer.[207,220,221,222,223] is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.8 million new cases in 2018. The top 25 countries with the highest rates of colorectal cancer in 2018 are given in the tables below.

Colorectal cancer.[224,225] (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world, and its burden is expected to increase by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030.

The Continuous Update Project Panel judged there was strong evidence that consuming processed meat, red meat and alcoholic drinks, greater body fatness and adult attained height increase the risk of colorectal cancer. There was also strong evidence that physical activity is protective against colon cancer specifically and that wholegrains, foods containing dietary fibre, dairy products and calcium supplements decrease the risk of colorectal cancer.

Colorectal cancer is considered one of the clearest markers of epidemiological and nutritional transition, with incidence rates of this cancer – together with other cancers linked to Western lifestyles – increasing as previous high rates of infection-related cancers decline in countries that are undergoing rapid societaland economic changes

Colon Cancer Cell Line[226,227] (HCT15)
It is a continuous culture, grown as monolayerepithelial-like morphologySPECIES: human
TISSUE/ORGAN: colon

TUMOR: colorectal adenocarcinoma/tumorigenic in nude mice.

1.2 Plant Profile
Scientific classification
Kingdom: Plantae (Unranked): Angiosperms (Unranked): Eudicots (Unranked): Asterids **Order:** Lamiales
Family: Bignoniaceae **Genus:** *Tecoma* Species: ***T. capensis***
Binomial name: ***Tecoma capensis*** *(Thunb.) Lindl.*

Synonyms: *Tecomaria capensis*

Local Names
Africaans (kaapse kanferfoelie); English (tecoma, kaffir honeysuckle, cape honeysuckle); Xhosa(icakatha); Zulu (uminyane, ugcangca, uchacha)

Figures 13: *Tecomaria capensis* flowers.

Figures 14: *Tecomaria capensis* leaves.

Botanic Description
Tecomaria capensis is an evergreen scrambler to small tree with a roundish crown. Bark pale brown, lenticelled with longitudinal furrows on old stems. Leaves opposite, unevenly compound, up to 13 cm long, with 2-5 pairs of leaflets, terminal leaflet largest, margins coarsely toothed, glossy green above. Fruit a narrow, flat pod-like capsule up to 13 cm long.

Seeds with large papery wings. There are 3 garden cultivars; “coccinea” with light red flowers on a bushy plant, “lutea” with bright yellow flowers on a spreading bush and “salmonii” with salmon-coloured flowers. The genus Tecomaria is monotypic and has affinities with Tecoma.

Biology
The cape honeysuckle is dioecious and evergreen; usually flowering after rains from June-November and fruiting from October-February. Pollinated by birds and insects. Calyx 5-lobed, much shorter than corollatube. Corolla bilabiate, tube curved, narrowly funnel-shaped; one lip 2-lobed; all lobes elliptic, obtuse. Stamens didynamous, inserted in lower part of corolla-tube, exserted; filaments terete; anthers 2- thecous with thecae at length separating. Style terete, exserted, with elliptic, 2-lobed stigma.

Ecology
T. capensis occurs on forest margins but more commonly along drainage lines in dense woodland. Grows well in moist areas and in dry scrub and woodland.

Biophysical Limits
Altitude: 0-1 200 m

Mean annual temperature: 22-26 deg.C Mean annual rainfall: 750-1 750 mm
Soil type: Grows in a variety of soils types.

Documented Species Distribution
Exotic range: Lesotho, Mozambique, South Africa, Swaziland, Tanzania

Native range: India, Kenya, Singapore, Spain, United Kingdom

Products
Fodder: Foliage readily browsed by stock and game.
Apiculture: The flowers are rich in nectar thus attract a number of pollinators especially sunbirds and bees.
Fuel: The plant can be used as firewood.
Medicine: Powdered bark used for treatment of fever, pneumonia and stomach troubles, also rubbed on bleeding gums to promote blood clotting. Leaf decoction used for diarrhoea and for intestinal inflammation. Believed to ease pain and produce sleep.

Services
Erosion control: The cape honeysuckle protects surrounding soil from erosion
Apiculture: The cape honeysuckle is a rich source of sugar.
Shade or shelter: Unpruned trees provide adequate shade
Soil improver: The leaf litter on decomposition improves soil fertility.
Ornamental: A prized ornamental with a showy and profuse bloom, cultivated in several gardens, parks and arboreta.
Boundary or barrier or support: The cape honeysuckle is a wonderful fencing plant with good regrowth ability after pruning and normally dense and colourful foliage over a long time.

Tree Management
The cape honeysuckle must be pruned, to stay attractive in gardens and enhance flowering. The plant grows fast usually flowering in the second year. Growing should be done in semi shade or full sun conditions. The plant is frost tender and should be protected during the first two winters.

Germplasm Management
Seed wings removal must be done before planting.

Pests and Diseases
The pathogenic fungus *Phytophthora palmivora* has been detected on *T. capensis* leaves.

Figures 15: Whole plant of *Tecomaria capensis.*

2. Review of Literature

Flavonoids as antioxidants from flower of *tecomaria capensis lindl var. Aurea*.[228]
In this study phytochemical investigation of the methanolic extract from the flowers of *Tecomaria capensis* Lindl var. aurea led to the isolation of ferulic **1**, rutin **2**, luteolin-7-O-β-D- glucuronopyranoside **3**, apigenin-7-O-β-D- glucuronopyranoside **4** and luteloin -7-O-(6-O-E-p. coumaroyl) β -D- glucopyranoside **5**. This is the first report for the isolation of these compounds from this variety. Free radical, superoxide radical scavenging and deoxyribose assay of the isolated compounds where evaluated in vitro and the antioxidant effects were compared with the same dose of commercial and standard antioxidants such as vitamin C and BHA [butylated hydroxyanisol].

Antibacterial and Antifungal Activity of Ethanol Extract of Different Parts of Medicinal Plants in Jordan.[229]
In this study agar diffusion assay and Minimum Inhibitory Concentration (MIC) determinations, in vitro were used to evaluate antimicrobial activity of plant extracts against nine bacteria and four fungi. The ethanolic extracts of plant Tecoma capensis Thunb. Lindl (Bignoniaceae) were assayed. T. capensis flower extract exhibited strong antifungal activity (17 mm and 0.5 mg MIC) and inhibited moderately methicillin resistant Staphylococcus aureus which is reported for the first time. This plant extract showed interesting antimicrobial activity against bacteria and fungi.

Evaluation of wound healing activity of *Tecomaria capensis* leaves[230]
In this study they evaluated the potential of wound healing activity of *Tecomaria capensis* leaves extract (TCLE) using different models in rats. (a) Excision wound model, (b) Incision wound model and (c) Dead space wound model. TCLE 5% and 10% ointment were applied topically in excision wound model and incision wound model. TCLE 200 and 400 $mgkg^{-1}$ were given orally in dead space wound model. It improved healing in excision wound model, increased breaking strength of tissue in incision wound model, and increased granuloma breaking strength and hydroxyproline content in dead space wound model. These results showed that TCLE presents significant wound healing activity.

In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa[231]
In this study they reported that in vitro antiplasmodial activity of Tecomaria capensis (Thunb.) Lindl. Theplant extract was tested for in vitro activity against a Plasmodium falciparumstrain D10 using the parasite lactate dehydrogenase (pLDH) assay. Leaves of tecomaria capensis Solvent DCM/MeOH (1:1), %Yield - 0.1, IC50 - 11.6(µg/ml). The first time to possess in vitro antiplasmodial activity.

Evaluation of Antioxidant Activity of Tecomaria Capensis Leaves[232]
In this study antioxidant activity of methanolic Tecomaria capensis leaves extract (TCLE) find out by using different in-vitro models. It includes Free radical scavenging activity of 1,1-Diphenyl-2-picryl- hydrazil (DPPH), Ferric reducing antioxidant power, Total flavonoid content and Total phenolic content. Plant contains much amount of Phenolic compounds and Flavonoids. Plant shows significant antioxidant activity.

Iridoid and phenylpropanoid glucosides from *Tecoma capensis*[233]
Leaves of *Tecoma capensis* contain, together with tecomoside, large quantities of it's benzoic and cinnamic esters. A novel glucoside was isolated and, by spectroscopic and chemical data, characterized as 7-*O*-(*p*-methoxy) benzoyl tecomoside. Flowers of *T. capensis* contain only tecomoside, together with two phenylethanoid-derived glucosides, cornoside and it's rearranged aglycone, halleridone, and rengioside B.

Evaluation of antipyretic activity of methanolic *Tecomaria capensis* leaves extract[234]
In this study they evaluated the potential Antipyretic activity of methanolic *Tecomaria capensis* leaves extract using different models in rats. Materials and methods: Anyretic activity was evaluated using brewer's yeast induced pyrexia model in rats. Methanolic *Tecomaria capensis* leaves extract were given at dose of 100, 200 and 500 mg/kg p.o.

Results: Results demonstrated that the methanolic *Tecomaria capensis* leaves extract at the doses of (100, 200 and 500 mg/kg p.o.) significantly decreased the rectal temperature of the rats.

3. Aim of The Study
Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India since long times. The value of medicinal plants, herbs and spices as herbal remedies is being lost due to lack of awareness, and deforestation. The result is many valuable medicinal herbs are becoming rare and precious information is lost. Less pollution we make, more ecological balance we maintain, will add to happiness of humankind. Preserve the knowledge of medicinal plants, herbs, spices and herbal remedies, which humankind has received from the past generations, for posterity.

The herbal medicines are effective in the treatment of various life threatening diseases. Very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. The detailed investigation and documentation ofplants used in total health traditions and pharmacological evaluation can lead to the development of invaluable plant drugs for many dreaded diseases.

Therefore, based on the above facts, the present study has been undertaken with the main objective

- Literature review
- Selection, authentication and preparation of crude extracts
- Study the preliminary pharmacognostical, physicochemical and phyto chemicalparameters on selected medicinal plant crude extract
- *In-vitro* Anticancer activity of ethyl acetate flower extract of *Tecomaria capensis* on Colon cancer cell line (HCT15)

➢ Literature survey.

4. Plan of Work
➢ Selection and authentication of medicinal plant species.
➢ Preliminary pharmacognosy study
❖ Organoleptic observation
❖ Microscopical study
➢ Preliminary physico chemical parameter studies
❖ Moisture content
❖ Foreign matter content
❖ Extract value
❖ Ash value
➢ Preparation of different crude extracts by successive solvent through continues hot percolations method.
➢ Preliminary phyto chemical screening.
➢ *In-vitro* pharmacological screening of anti-cancer activity on Colon cancer cellline (HCT15) cell line of *Tecomaria capensis* by using ethyl acetate extraction

5. Materials and Methods
5.1. Identification and Authentification of Selected Plant Species
The leaves of *Tecomaria capensis* (Thunb.) Spach was collected from Guntur district, A.P and it was authenticated by professor Dr.S.M.Khasim, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The specimen (No: ANU/00129/2009/AP) was deposited in the department of botany and microbiology for future reference.

5.2. Collection of Plant Material
Fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The

coarse powder was passed through sieve no. 60#. Care was taken to select healthy plants and for normal organs.

5.3 Macroscopical Study

As per standard procedure matured 25 leaves were taken for the evaluation of morphology of leaves and studied various parameters such as length, width, margin, apex, surface, colour, odour, taste, type, base, midrib and size. By visual method the organoleptic characteristics of leaf was found.

5.4 Material and Methods

5.4.1 Materials

Chemicals: Ethyl acetate, Dragendroffs reagent, Mayer's reagent, sodium picrate, conc. sulphuric acid, ammonia, dilute Hcl, lead acetate, ferric chloride.

Glassware: beaker, glassrod, roundbottom flask, microtiter plate, multi channeled pipette,

Instruments: soxhlet apparatus, distilation apparatus, heating mantle,Microtiter plate reader

5.4.2 Methods

5.4.2.1 Preparation of Extracts

The techniques commonly used in the field of phytochemistry are extraction, isolation, and structural elucidation of natural products, as well as chromatographic techniques. The solvent extraction of any botanical materials may yield very less quantity of volatile oils and a large yield of non-volatile components like resins, pigments, waxes and fatty acids.

Ethyl Acetate Extract

This extract was prepared by using soxhlet apparatus. About 150gm of dried flower powder was taken in a muslin cloth bag. The purified Ethyl acetate was passed through the tube where the powder bag was kept. The Ethyl acetate will pass through siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapors containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing extract is transferred to a beaker and is allowed to evaporate in a water bath. This concentrated Ethyl acetate extract is used for further studies.

5.4.2.2 *In-Vitro* Pharmacological Screening Mtt Assay Method:[235]

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high through put screening. Measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

The MTT method of cell determination is most useful when cultures are prepared in multiwell plates. For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells.

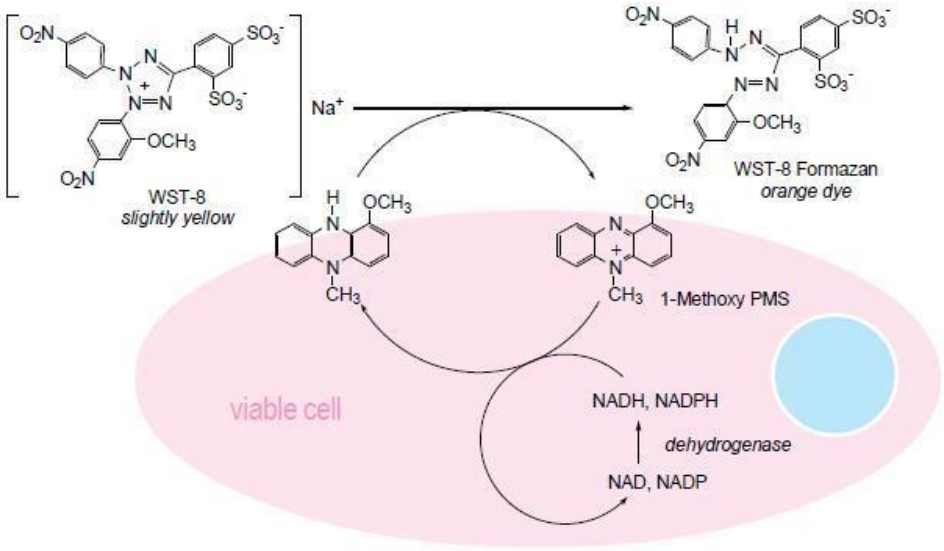


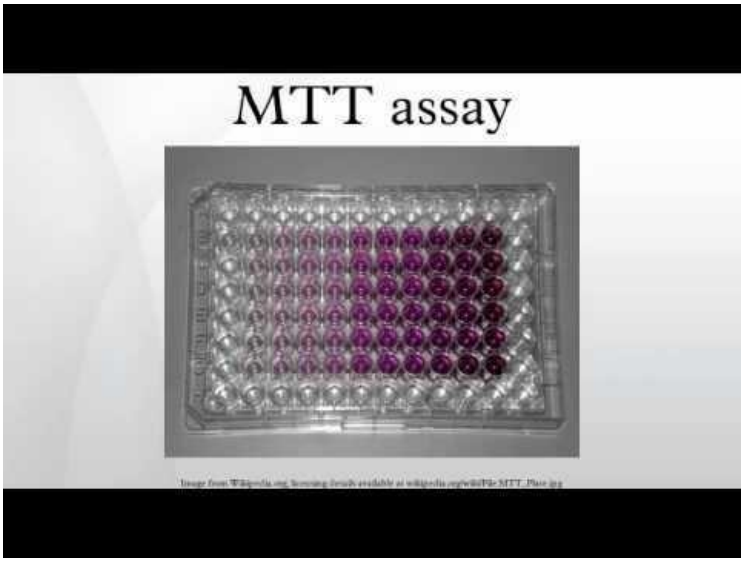
Figure-16:

Thiazolyl Blue Tetrazolium Bromide (MTT) Product Number M 2128 Storage Temperature 2-8 oC.

Equipments Used:[284]

- Microtiter plate reader with 650- and 570-nm Filters.
- Inverted microscope.
- Multi-channel pipette.
- 37°C incubator.
- Laminar flow hood.
- Microtiter plate (flat-bottomed).
- Sterile tubes (5 mL).
- Serological pipettes.
- Sterile pipette tips.

Figure-17: Multi Channeled Pipette.



Microtiter plate

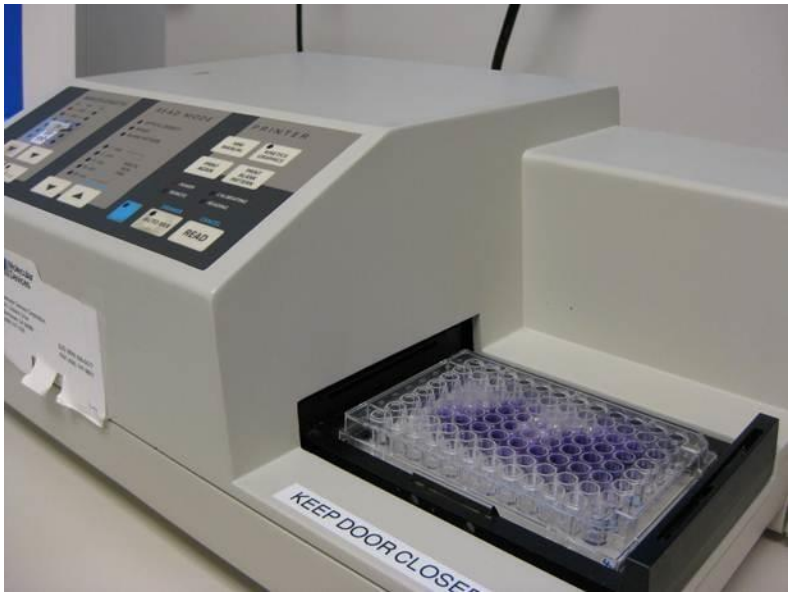


Figure-18: Microtiter plate reader with 650- and 570-nm Filters.

Preparation Instructions

MTT is soluble in water (10 mg/ml), ethanol (20 mg/ml) and is also soluble in buffered salt solutions and culture media (5 mg/ml). Reconstituted MTT solution is stable for at least 6 months when stored at -0^{o}C. Storage at 4^{o}C for more than four days will result in decomposition and will yield erroneous results.

MTT Solution

5 mg/ml MTT in PBS. Solution must be filter sterilized after adding MTT.

MTT Solvent

4 mM HCl, 0.1% Nondet P-40 (NP40) all in isopropanol.

Important Assay Notes

1. Remove cultures from incubator into laminar flow hood or other sterile working area.
2. Aseptically add MTT SOLUTION in an amount equal culture volume.
3. Incubation times should be consistent when making comparisons.
4. If cells are attached to culture vessel growth surface, remove and dispose of the culture fluid. Add MTT SOLVENT in an amount equal to the original culture volume. Solvent volumes may vary but the final volumes should be consistent to facilitate comparison.
5. If cells are not attached or loss of MTT formazan occurs if culture fluid is removed, add MTT SOLVENT
6. Gentle stirring in a gyratory shaker will enhance dissolution. Occassionally, pipetting up and down (trituration) may be required to completely dissolve the MTT formazan crystals especially in dense cultures.

Note

For most tumor cells, hybridomas, and fibroblast cell lines, 5,000 cells per well to perform proliferation assays. 12 well plates need 1 ml of solution and have about 400,000 cells, 24 well plates have 0.5 ml and 200,000 cells at confluency.

Procedure

Short 96 well assay: EACH condition should be done in triplicate or more.

1. DAY ONE: Trypsinize one T-25 flask and add 5 ml of complete media to trypsinized cells. Centrifuge in a sterile
2. 15 ml falcon tube at 500 rpm in the swinging bucked rotor (~400 x g) for 5 min.
3. Remove media and re suspend cells to 1.0 ml with complete media.
4. Count and record cells per ml. Remember to remove the cells aseptically when counting.
5. DILUTE the cells (cv=cv) to 75,000 cells per ml. Use complete media to dilute cells.
6. Add 100 µl of cells (7500 total cells) into each well and incubate overnight.
7. DAY TWO: Treat cells on day two with agonist, inhibitor or drug.
8. If removing media, do very carefully. This is where most variation in data may occur.
9. Final volume should be 100 µl per well.
10. DAY THREE: Add 20 µl of 5 mg/ml MTT to each well. Include one set of wells with MTT but no cells(control).

All should be done aseptically

1. Incubate for 3.5 hours at 37oC in culture hood.
2. CAREFULLY Remove media. Do not disturb cells and do not rinse with PBS.
3. Add 150 µl MTT solvent.
4. Cover with tinfoil and agitate cells on orbital shaker for 15 min.
5. Read absorbance at 590 nm with a reference filter of 620 nm.

Storage: The MTT Reagent must be kept at 4C in the dark. The Detergent Reagent can be stored ateither 4C or ambient temperature. If the detergent reagent is kept at 4C, warm the bottle for 5 minutes at 37C and gently mix by inverting before use (avoid creating bubbles).

Table-3

Component	Volume	storage
MTT Reagent	25ml	4°C
Detergent Reagent	2 × 125 mL	Room Temp. or 4°C

Formulas used for calculation

$$\text{Cell viability}(\%) = \left[\frac{\left(\overline{A_{\text{sample}}} - \overline{A_{\text{blank}}}\right)}{\left(\overline{A_{\text{control}}} - \overline{A_{\text{blank}}}\right)}\right] \times 100\%$$

$$\text{Vialibity}(100\%) = \frac{\text{mean of absorbance value of treatment group}}{\text{mean absorbance value of control}} \times 100\%$$

In the present study (ethyl acetate) flowers extract of *Tecomaria capensis.*

5.8 Micro Biological Assay

I have examine in-vitro anticancer activity of ethyl acetate flower extract of *Tecomaria capensis on Colon cancer cell line (HCT15)*

Principle

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening (HTS). The MTT tetrazolium assay technology has been widely adopted and remains popular in academic labs as evidenced by thousands of published articles. The MTT substrate is prepared in a physiologically balanced solution, added to cells in culture, usually at a final concentration of 0.2 - 0.5mg/ml, and incubated for 1 to 4 hours. The quantity of formazan (presumably directly proportional to the number of viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. A reference wavelength of 630 nm is sometimes used, but not necessary for most assay conditions.

Viable cells with active metabolism convert MTT into a purple coloured formazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan, thus colour formation serves as a useful and convenient marker of only the viable cells. The exact cellular mechanism of MTT reduction into formazan is not well understood, but likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT. Speculation in the early literature involving specific mitochondrial enzymes has led to the assumption mentioned in numerous publications that MTT is measuring mitochondrial activity.

The formazan product of the MTT tetrazolium accumulates as an insoluble precipitate inside cells as well as being deposited near the cell surface and in the culture medium. The formazan must be solubilized prior to recording absorbance readings. A variety of methods have been used to solubilize the formazan product, stabilize the colour, avoid evaporation, and reduce interference by phenol red and other culture medium components. Various solubilisation methods include using: acidified isopropanol, DMSO, dimethylformamide, SDS, and combinations of detergent and organic solvent. Acidification of the solubilizing solution has the benefit of changing the colour of phenol red to yellow colour that may have less interference with absorbance readings. The pH of the solubilisation solution can be adjusted to provide maximum absorbance if sensitivity is an issue; however, other assay technologies offer much greater sensitivity than MTT.

The amount of signal generated is dependent on several parameters including: the concentration of MTT, the length of the incubation period, the number of viable cells and their and metabolic activity. All of these parameters should be considered when optimizing the assay conditions to generate a sufficient amount of product that can be detected above background.

Cell culture and MTT assay

The Colon cancer cell line (HCT15) were plated separately using 96 well plates with the concentration of 1×104cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO2 incubator at 37˚C with 5% CO2. The cells were washed with 200 µL of 1X PBS, then the cells were treated with various test concentration of compound in serum free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37˚C for 4 h using CO2 incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals was dissolved with 100 µL of DMSO and thoroughly mixed. The development of colour intensity was evaluated at 570nm. The formazan dye turns to purple blue colour. The absorbance was measured at 570 nm using microplate reader.

RESULTS AND DISCUSSION

The herbal medicines are effective in the treatment of various life threatening diseases. Very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. The detailed investigation and documentation of plants used in total health traditions and pharmacological evaluation can lead to the development of invaluable plant drugs for many dreaded diseases.

Macroscopical Characteristics

Leaf - Isobilateral,
Shape - Ovate,

Size
Length - 6 - 7.5 cm,
Width - 2.5 – 3.5 cm,
Texture - Glabrous,
Apex - Acute,
Margin - Serrated,
Base - Decurrent,
Petiole - Expetiolate,
Surface - Glossy,
Colour:
Outer - Dark green,
Inner - Light green,
Venation - Pinnate,
Odour - Characteristic

Microscopy Characteristics

Transverse section of leaves of *Tecomaria Capensis*
Type: - Isobilateral, convex type of leaf.

Upper epidermis

It is of single layer non-lignified rectangular shape of cells arranged transversally.

Lower epidermis

It is of single layer non-lignified rectangular shape of cells arranged transversally.

Trichomes

Both covering as well as glandular trichomes are present. Covering trichomes are present just above the epidermal layer. Glandular trichomes are present in more number in the midrib portion.

Stomata:- Anamocytic stomata.

Palisade cells

Below the upper epidermis and above lower epidermis palisade cells are longitudinally compactly arranged and they are terminated up to the lamina portion or mesophyll portion.

Mid Rib

Spongy parenchyma

Polygonal, loosely arranged cells with intracellular spaces and non lignified.

Vascular Bundles

Isobilateral vascular bundles are seen in central portion of midrib. It contains xylem (lignified) Phloem and surrounded with phloem parenchyma. Below the upper epidermis and above the lower epidermis encircled with chollenchyma.

Chollenchyma

Contains thick walled non-lignified polygonal shape of cells.

Powder Microscopy

Trichomes

Multicellular, non lignified slightly curved with central lumen.

Xylem vessels

Lignified, narrow, sieve tube like bundles of vessels.

Epidermal cells

Non-lignified rectangular shaped cells arranged transversally.

Calcium Oxalate Crystals

Mesophyll region contains calcium oxalate crystals. Isolate or cluster of prism of calcium oxalate crystals.

Figure-19: Leaves of *Tecomaria capensis*T.S of *Tecomaria capensis.*

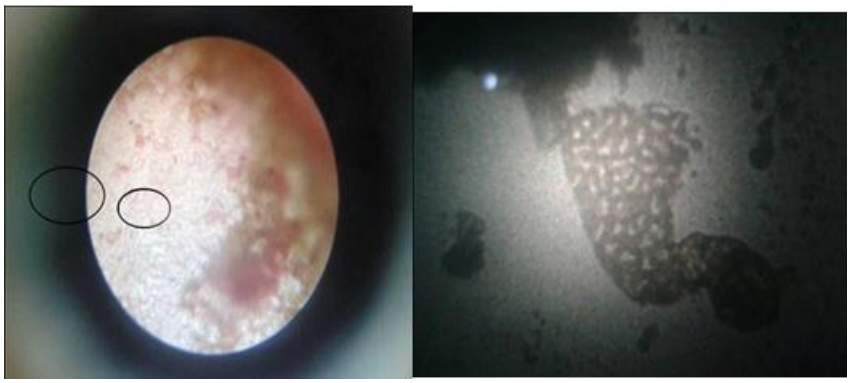


Figure-20Anamocytic stomataSpongy parenchymatous cells.

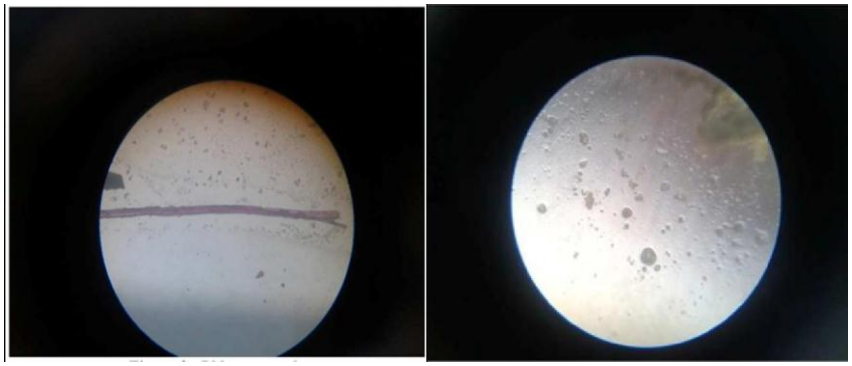


Figure 21: Phloem vessels Clusters of calcium oxalate crystals.

Physicochemical Constituents

Determination of Physicochemical constituents are performed as per the standard protocol followed in the Ayurvedic pharmacopoeia. The values are tabulated in **Table 4 and 5.**

Table-4: The foreign matter adulterated in one gram powder was found to be 2%.

DIFFERENT EXTRACTIVE VALUES

Extractive value	Values
Alcohol soluble extraction	0.25 gm
Water solble extraction	0.36gm
Ether soluble extraction	0.04gm

Table-5: Percentage Yield of *Tecomaria Capensis*extracts

DIFFERENT ASH VALUES OF *TECOMARIA CAPENSIS* LEAVES

ASH VALUES	In grams
Total ash value	0.93
Acid insoluble ash value(dil.Hcl)	0.01
Sulphated ash value (H_2SO_4)	0.06
Water soluble ash value(H_2O)	0.05

Table-6: Preliminary Phyto Chemical Screening

Sr. No.	Solvents	Nature of extract	Color	% yield
1	Pet-ether	Semisolid	Dark yellow	0.35
2	N-Hexane	Semisolid	Dark yellow	0.13
3	Chloroform	Semisolid	Dark Green	0.20
4	Ethyl acetate	Semisolid	Dark Green	0.70
5	Ethanol	Semisolid	Dark Green	0.52
6	Aqueous	Semisolid	Dark brown	0.25

% yield of different leaves extracts of *Tecomaria capensis* shown in Table no 6.

Table-7:

Phyto constituents	Pet. Ether	n-hexane	Chloroform	Ethyl acetate	Ethanol	Water
Alkaloids	--	--	–	--	--	--
Flavinoids	--	--	++	++	++	++
Cardiac Glycosides	++	++	++	++	++	--
Saponin Glycosides	++	++	++	++	--	--
Coumarin Glycosides	--	--	--	++	++	--
Tannins	--	--	--	--	++	--
Steroids and terpinoids	--	++	++	++	++	--
Carbohydrates	--	--	--	++	--	--
Protein	--	--	–	++	++	--
Inulin	--	--	–	--	++	++
Volatile oil	++	++	++	++	++	--
Waxes	--	--	--	--	–	--
Mucilage	--	--	--	--	++	++

++ Present, -- Absent TABLE NO: 4

Phytochemical studies of*Tecomaria Capensis* leaves revealed the presence of cardiac glycosides, saponin glycosides and volatile oils in pet. Ether extract. In n-hexane extract cardiac glycosides, steroids and triterpinoids and volatile oils are present. In chloroform extract flavanoids, cardiac glycosies, saponin glycosides, steroids, triterpenoids and volatile oils are present. In ethyl acetate extract flavanoids,cardiac glycosides, coumarin glycosides, saponin glycosides, steroids, terpenoids, carbohydrates, proteins and volatile oils are present. In ethanolic extract alkaloids, flavanoids, cardiac glycosides, coumarin glycosides, tannins, steroids, terpinoides, proteins, inulin, volatile oil and mucilage are present. Inwater extract flavanoid, inulin and mucilage are present. In ethanol and ethyl acetate extracts maximum phyto chemical constituents are been present.

Pharmacological Screening [Colon cancer cell line (HCT15)]
The below given table shows the OD value at 570nmSample code: Tecomaria capensis 103.

Tested concentration(µg/ml)	OD at 570nm(triplicate values)		
25	0.351	0.357	0.355
50	0.337	0.34	0.338
100	0.32	0.33	0.317
250	0.31	0.315	0.317
500	0.298	0.3	0.302
Control	**0.361**	**0.365**	**0.368**

Table-8: The below given table shows the %ViabilityTable-9.

Tested concentration(µg/ml)	OD at 570nm(triplicate values)		
25	0.351	0.357	0.355
50	0.337	0.34	0.338
100	0.32	0.33	0.317
250	0.31	0.315	0.317
500	0.298	0.3	0.302
Control	**0.361**	**0.365**	**0.368**

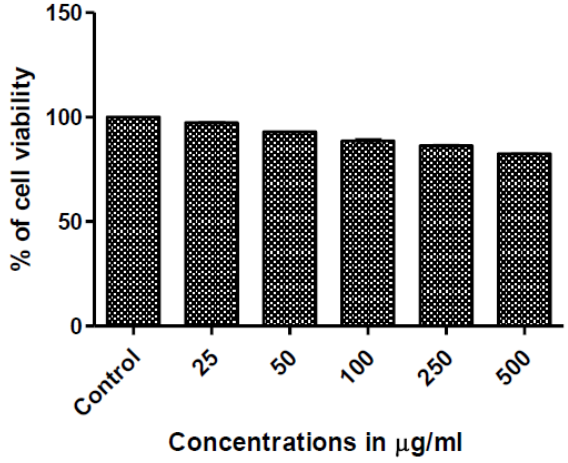


Figure-22

25 µg/ml50 µg/ml

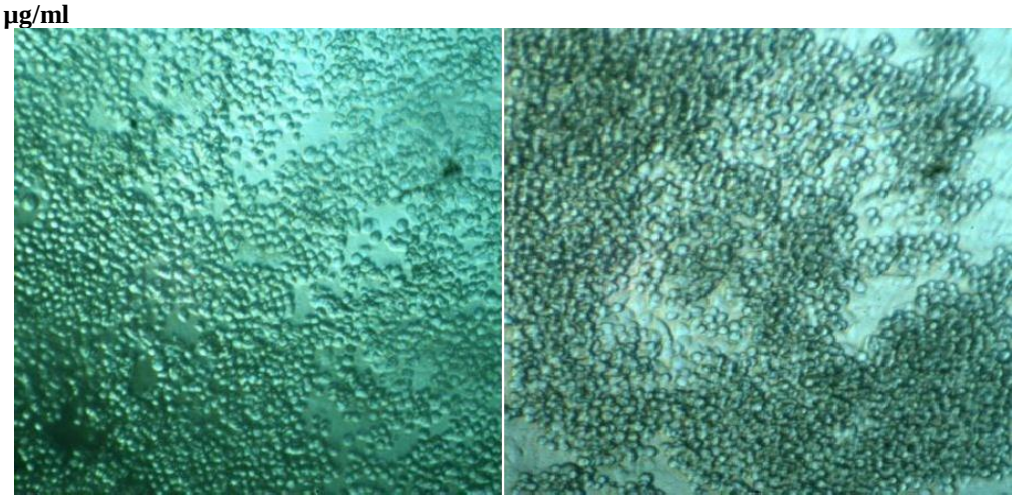
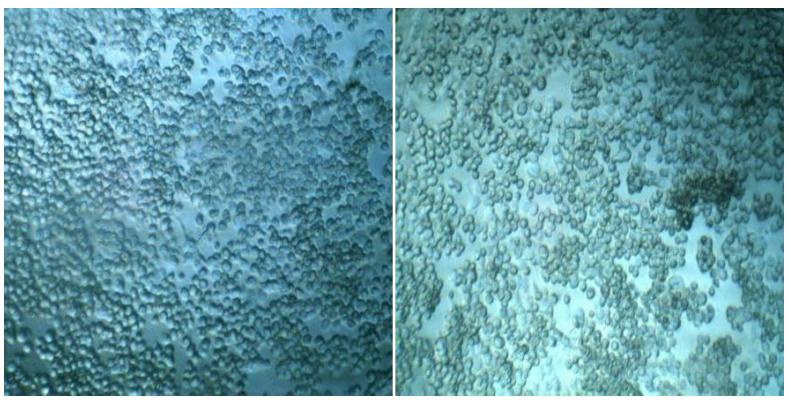
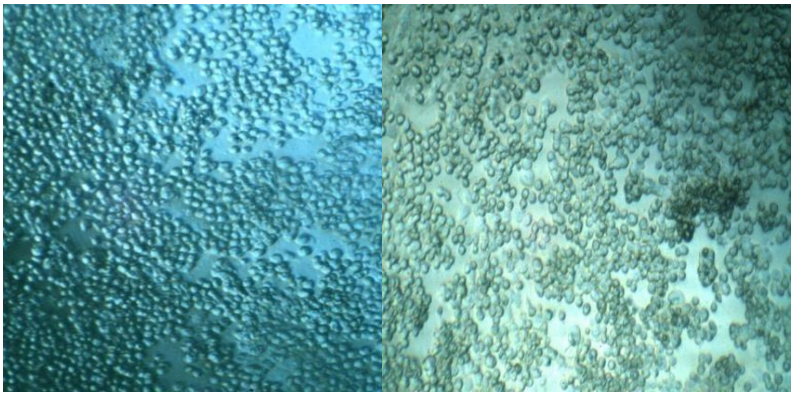


Figure-22 % of cell viability



100 µg/ml250 µg/m



500 µg/mlControl

Comparison of different concentration of samples with controlFigure-23

In-vitro cytotoxic activity of *Tecomaria capensis* ethyl acetate extractat various concentration against Colon cancer cell line (HCT15) cancer cell lines were studied using MTT assay. Anti tumour activity of *Tecomaria capensis* ethyl acetate extractat various concentrations against Colon cancer cell line (HCT15) cancer cell lines was represented in Figure 22. With increase in concentration of *Tecomaria capensis* ethyl acetate extractfrom 25, 50, 100, 250, 500 µg/ml. documents reduced percentage of cell viability respectively. Then the percentage of cell density has been decreased evident the cell death.

SUMMARY AND CONCLUSION

India is one of the largest producers of medicinal herbs in the world. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development. Nature has provided a complete store-house of remedies to cure all ailments of mankind by providing us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect. Research on medicinal plants is an important fact of biochemical research in India because of several reasons.

In the present study, the attempt is made to identify the active constituent responsible for the pharmacological activity. *Tecomaria capensis* flowers were extracted in ethyl acetate. In phytochemical investigation phytoconstituents like flavonoids, cardiac glycosides, saponin glycosides, tannins, steroids, terpenoids, proteins, inulin, volatile oil, and mucilage are found to be present.

The results described here clearly confirmed the anti-cancer properties of *Tecomaria capensis* ethyl acetate extract.Present study infers that the *Tecomaria capensis* ethyl acetate extract exhibit effective antitumor activity and seems to have no side effects. They are less cost effective, easy in production andpurification. In future it can be recommended to the patients as a effective therapeutic tool in form of food or drug. Further research need to be explored to study the bioactive compounds of *Tecomaria capensis* ethyl acetate extract and for the successful implication of them as a potent therapeutic tool against cancer.

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