Green Tea: A Promising Anticancer Agent for Cell Carcinoma.

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Abstract– Cancer is characterized by proliferation of cells that have managed to evade central endogenous control mechanisms. There are many claims on the Internet and in publications about substances that treat cancer (for example, broccoli, grapes, ginseng, soybeans, green tea, aloe vera, and lycopene and treatments like acupuncture, vitamins, and dietary supplements). Almost every physician suggests that a balanced diet and good nutrition will help an individual combat cancer. Although some of these treatments may help reduce symptoms, there is no good evidence they can cure any cancers. Patients are strongly recommended to discuss any home remedies or alternative treatments with their cancer doctors before beginning any of these.

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells anywhere in a body.

There are over 200 types of cancer. Anything that may cause a normal body cell to develop abnormally potentially can cause cancer; general categories of cancer-related or causative agents are as follows: chemical or toxic compound exposures, ionizing radiation, some pathogens, and human genetics.

Cancer symptoms and signs depend on the specific type and grade of cancer; although general signs and symptoms are not very specific the following can be found in patients with different cancers: fatigue, weight loss, pain, skin changes, change in bowel or bladder function, unusual bleeding, persistent cough or voice change, fever, lumps, or tissue masses.

Although there are many tests to screen and presumptively diagnose cancer, the definite diagnosis is made by examination of a biopsy sample of suspected cancer tissue. (1)

Treatment protocols vary according to the type and stage of cancer. Most treatment protocols are designed to fit the individual patient's disease. However, most treatments include at least one of the following and may include all: surgery, chemotherapy, and radiation therapy.

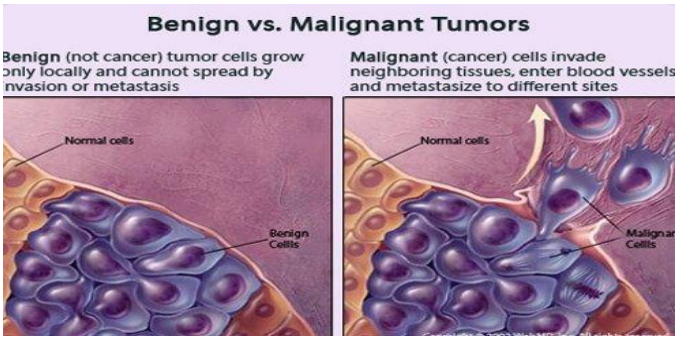
There are many listed home remedies and alternative treatments for cancers but patients are strongly recommended to discuss these before use with their cancer doctors.

In the most basic terms, cancer refers to cells that grow out-of-control and invade other tissues. Cells become cancerous due to the accumulation of defects, or mutations, in their DNA. Certain:

1. inherited genetic defects (for example, BRCA1 and BRCA2 mutations),
2. infections,
3. environmental factors (for example, air pollution), and
4. poor lifestyle choices -- such as smoking and heavy alcohol use -- can also damage DNA and lead to cancer

Most of the time, cells are able to detect and repair DNA damage. If a cell is severely damaged and cannot repair itself it undergoes so-called programmed cell death or apoptosis. Cancer occurs when damaged cells grow, divide, and spread abnormally instead of self-destructing as they should.

Cancer is not confined to humans; animals and other living organisms can get cancer. Cancer cells can break away from this original mass of cells, travel through the blood and lymph systems, and lodge in other organs where they can again repeat the uncontrolled growth cycle. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastasis.



The incidence of cancer and cancer types are influenced by many factors such as age, gender, race, local environmental factors, diet, and genetics. Consequently, the incidence of cancer and cancer types vary depending on these variable factors.

For example, the World Health Organization (WHO) provides the following general information about cancer worldwide:
1-Cancer is a leading cause of death worldwide. It accounted for 8.2 million deaths (around 22% of all deaths not related to

communicable diseases; most recent data from WHO). Lung, stomach, liver, colon, and breast cancer cause the most cancer deaths each year.
2-Deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030 (about a 70% increase). Different areas of the world may have cancers that are either more or less predominant than those found in the U.S. One example is that stomach cancer is often found in Japan, while it is rarely found in the U.S. This usually represents a combination of environmental and genetic factors.

What are risk factors and causes of cancer?

Anything that may cause a normal body cell to develop abnormally potentially can cause cancer. Many things can cause cell abnormalities and have been linked to cancer development. Some cancer causes remain unknown while other cancers have environmental or lifestyle triggers or may develop from more than one known cause. Some may be developmentally influenced by a person's genetic makeup. Many patients develop cancer due to a combination of these factors.
The following is a listing of major causes and is not all-inclusive as specific causes are routinely added as research advances:
1. Chemical or toxic compound exposures: Benzene, asbestos, nickel, cadmium, vinyl chloride, benzidine, -nitrosamines, tobacco or cigarette smoke (contains at least 66 known potential carcinogenic chemicals and toxins), asbestos, and aflatoxin.
2. Ionizing radiation: Uranium, radon, ultraviolet rays from sunlight, radiation from alpha, beta, gamma, and X-ray-emitting sources.
3. Pathogens: Human papillomavirus (HPV), EBV or Epstein-Barr virus, hepatitis viruses B and C, Kaposi's sarcoma-associated herpes virus (KSHV), Merkel cell polyomavirus, Schistosoma spp., and Helicobacter pylori; other bacteria are being researched as possible agents.
4. Genetics: A number of specific cancers have been linked to human genes and are as follows: breast, ovarian, colorectal, prostate, skin, and melanoma; the specific genes and other details are beyond the scope of this general article so the reader is referred to the National Cancer Institute for more details about genetics and cancer.

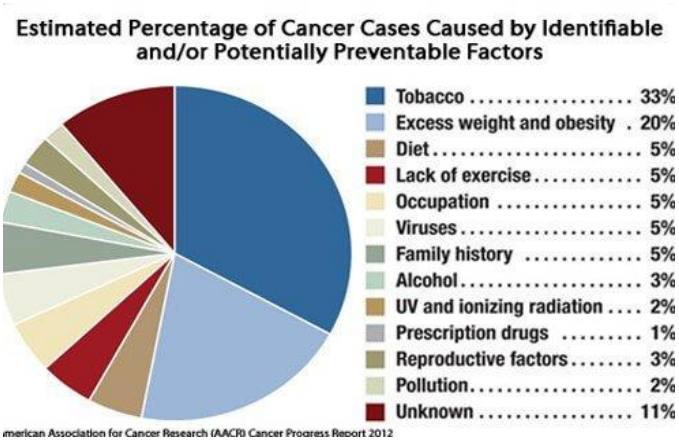
It is important to point out that most everyone has risk factors for cancer and is exposed to cancer-causing substances (for example, sunlight, secondary cigarette smoke, and X-rays) during their lifetime, but many individuals do not develop cancer. In addition, many people have genes that are linked to cancer but do not develop it. Why? Although researchers may not be able to give a satisfactory answer for every individual, it is clear that the higher the amount or level of cancer-causing materials a person is exposed to, the higher the chance the person will develop cancer. In addition, people with genetic links to cancer may not develop it for similar reasons (lack of enough stimulus to make the genes function).

In addition, some people may have a heightened immune response that controls or eliminates cells that are or potentially may become cancer cells. There is evidence that even certain dietary lifestyles may play a significant role in conjunction with the immune system to allow or prevent cancer cell survival. For these reasons, it is difficult to assign a specific cause of cancer to many individuals.

Recently, other risk factors have been added to the list of items that may increase cancer risk. Specifically, red meat (such as beef, lamb, and pork) was classified by the International Agency for Research on Cancer as a high-risk agent for potentially causing cancers; in addition, processed meats (salted, smoked, preserved, and/or cured meats) were placed on the carcinogenic list. Individuals that eat a lot of barbecued meat may also increase risk due to compounds formed at high temperatures.

Other less defined situations that may increase the risk of certain cancers include obesity, lack of exercise, chronic inflammation, and hormones, especially those hormones used for replacement therapy. Other items such as cell phones have been heavily studied. Proving that a substance does not cause or is not related to increased cancer risk is difficult.

Symptoms and signs of cancer depend on the type of cancer, where it is located, and/or where the cancer cells have spread. (2)



The American Cancer Society describes seven warning signs and/or symptoms that cancer may be present, and which should prompt a person to seek medical attention. The word CAUTION can help your rem
1. Change in bowel or bladder habits
2. A sore throat that does not heal
3. Unusual bleeding or discharge (for example, nipple secretions or a "sore" that will not heal that oozes material)
4. Thickening or lump in the breast, testicles, or elsewhere
5. Indigestion (usually chronic) or difficulty swallowing
6. Obvious change in the size, color, shape, or thickness of a wart or mole
7. Nagging cough or hoarseness ember these.

Anyone with these signs and symptoms should consult their doctor; these symptoms may also arise from noncancerous conditions.

What are the different types of cancer?

There are over 200 types of cancer; far too numerous to include in this introductory article. This list is expanded below to list more specific types of cancers found in each general category; it is not all inclusive and the cancers listed in quotes are the general names of some cancers:

1-Carcinoma: Cancer that begins in the skin or in tissues that line or cover internal organs.

2-Sarcoma: Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

3-Leukemia: Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

3-Lymphoma and myeloma: Cancers that begin in the cells of the immune system.

4-Central nervous system cancers: Cancers that begin in the tissues of the brain and spinal cord.

Not included in the above types listed are metastatic cancers; this is because metastatic cancer cells usually arise from a cell type listed above and the major difference from the above types is that these cells are now present in a tissue from which the cancer cells did not originally develop.

What specialists treat cancer?

A doctor who specializes in the treatment of cancer is called an oncologist. He or she may be a surgeon, a specialist in radiation therapy, or a medical oncologist. The first uses surgery to treat the cancer; the second, radiation therapy; the third, chemotherapy and related treatments. Each may consult with the others to develop a treatment plan for the particular patient.

How do health care professionals diagnose cancer?

Some cancers are diagnosed during routine screening examinations. These usually test that is routinely done at a certain age. A physical exam and medical history, especially the history of symptoms, are the first steps in diagnosing cancer. In many instances, the medical caregiver will order several tests, most of which will be determined by the type of cancer and where it is suspected to be located in or on the person's body. Also, most caregivers will order a complete blood count, electrolyte levels, and, in some cases, other blood studies that may give additional information.

Imaging studies are commonly used to help physicians detect abnormalities in the body that may be cancer. X-rays, CT and MRI scans, and ultrasound are common tools used to examine the body. Other tests such as endoscopy, which with variations in the equipment used, can allow visualization of tissues in the intestinal tract, throat, and bronchi that may be cancerous. In areas that cannot be well visualized (inside bones or some lymph nodes, for example), radionuclide scanning is often used. The test involves ingestion or IV injection of a weakly radioactive substance that can be concentrated and detected in the abnormal tissue.

What are cancer treatment options?

The cancer treatment is based on the type of cancer and the stage of the cancer. In some people, diagnosis and treatment may occur at the same time if the cancer is entirely surgically removed when the surgeon removes the tissue for biopsy.

Although patients may receive a unique sequenced treatment, or protocol, for their cancer, most treatments have one or more of the following components: surgery, chemotherapy, radiation therapy, or combination treatments (a combination of two or all three treatments).

Are there home remedies or alternative treatments for cancer?

There are many claims on the Internet and in publications about substances that treat cancer (for example, broccoli, grapes, ginseng, soybeans, green tea, aloe vera, and lycopene and treatments like acupuncture, vitamins, and dietary supplements). Almost every physician suggests that a balanced diet and good nutrition will help an individual combat cancer. Although some of these treatments may help reduce symptoms, there is no good evidence they can cure any cancers. Patients are strongly recommended to discuss any home remedies or alternative treatments with their cancer doctors before beginning any of these.

What is the role of green tea in treating cancer?

Green tea is made from Camellia sinensis and leaves through the oxidation process. It mainly originates in China and has been used traditionally throughout Asia. In the West, black tea has been used mostly, but green tea has become the most popular beverage throughout the world. It is also used as a raw material in cosmetics, health foods, and as an added ingredient in various beverages. Different varieties of green tea are available. The main differences between the varieties are due to harvesting time, production procedures, and horticulture. Drinking green tea has many positive effects on the body. It helps to nourish our five vital organs, among which the most important is the heart. It also has many qualities to help improve our state of mind (thus possibly reducing the consumption of alcohol), it acts as a stimulant, cures blotchiness, fulfills thirst, eliminates indigestion, cures beriberi disease, prevents fatigue, and improves kidney and brain function.

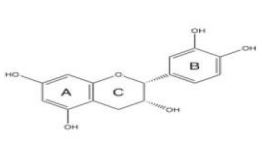
Green tea contains a 15% mixture of catechins. Of the four major catechins, (-)-epigallocatechin-3-gallate (EGCG) is the most prevalent. Green tea is implicated as a chemopreventive agent against the development of various tumors. The protective activity of green tea is generally assumed to be due to its free radical scavenging or a protective effect against gap junction intercellular communication (GJIC) inhibition.

However, little is known about the possible role of green tea in the development of renal cell carcinoma. Green tea catechins played an active chemopreventive role in chemical renal carcinogenesis in rats. However, the mechanisms of the anticarcinogenic activities of green tea are still elusive. Renal cell carcinoma is a malignant tumor of the kidney that accounts for about 3% of all malignancies.

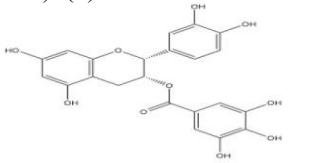
Acquired cystic kidney disease (ACKD) is commonly seen in patients who have undergone dialysis and its incidence increases with the duration of dialysis. Patients with ACKD have a propensity to develop adenocarcinomas. However, the etiology and prevention of ACKD transformation is still unclear. Gap junctions are membrane channels that mediate direct cell-cell communication properties by exchanging ions and metabolites less than 1.2 kD. These channels are formed of two hemichannels from the neighboring cells, and each hemichannel is composed of six proteins called connexins. The connexins belong to a multigene family with fourteen members cloned in the murine genome and differentially expressed among tissues. Gap junctions have been found to play important roles in the completion of embryonic development, maintenance of tissue homeostasis and regulation of normal cell growth. The disruption of GJIC may be important in carcinogenesis. The results of our previous studies have indicated that GJIC may play a role in renal carcinogenesis and that renal tumor tissues are characterized by low or aberrant connexin expression. (3)

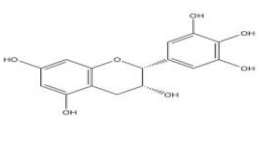
Epigallocatechin-3-gallate (EGCG), an active compound of green tea and its role in diseases cure and prevention has been proven. Its role in diseases management can be attributed to its antioxidant and anti-inflammatory properties. The anti-cancer role of this green tea compound has been confirmed in various types of cancer and is still being under explored. EGCG has been proven to possess a chemopreventive effect through inhibition of carcinogenesis process such as initiation, promotion, and progression. In addition, this catechin has proven its role in cancer management through modulating various cell signaling pathways such as regulating proliferation, apoptosis, angiogenesis and killing of various types of cancer cells. The additive or synergistic effect of epigallocatechin with chemopreventive agents has been verified as it reduces the toxicities and enhances the anti-cancerous effects. Despite its effectiveness and safety, the implications of EGCG in cancer prevention is certainly still discussed due to a poor bioavailability. Several studies have shown the ability to overcome poor bioavailability through nanotechnology-based strategies such as encapsulation, liposome, micelles, nanoparticles and various other formulation

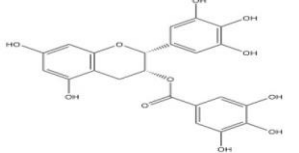
Green tea is composed of different chemical compounds, such as amino acids, vitamins, inorganic elements, carbohydrates, lipids, caffeine and tea polyphenols. Polyphenols constitute about 30% of the dry weight of green tea leaves and are the main compound responsible for its health promoting effects. Catechins form the major group of polyphenols found in green tea and comprise different molecules such as (−)-Epicatechin (EC), (−)-Epicatechin-3-gallate (ECG), (−)-Epigallocatechin (EGC), and (−)-Epigallocatechin-3-gallate (EGCG). (4)



(−)-Epicatechin (EC)


(−)-Epicatechin-3-gallate (ECG)





The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells:

Natural compounds are most favorable against cancer on the count of their anti-cancerous ability, easy to avail and efficient. Among natural compounds, polyphenols (flavonoids, catechin, hesperetin, flavones, quercetin, phenolic acids, ellagic acid, lignans, stilbenes, etc.) represent a large and diverse group used in the prevention and treatment of cancer. Natural flavonoids are derived from different plant sources and from various medicinal plants including Petroselinum crispum, Apium graveolens, Flemingia vestita, Phyllanthus emblica, etc. Natural flavonoids possess antioxidant, anti-inflammation, as well as anti-cancerous activities through multiple pathways, they induce apoptosis in breast, colorectal, and prostate cancers, lower the nucleoside diphosphate kinase-B activity in lung, bladder and colon cancers, inhibit cell-proliferation and cell cycle arrest by suppressing the NF-kB pathway in various cancers, etc. (5)

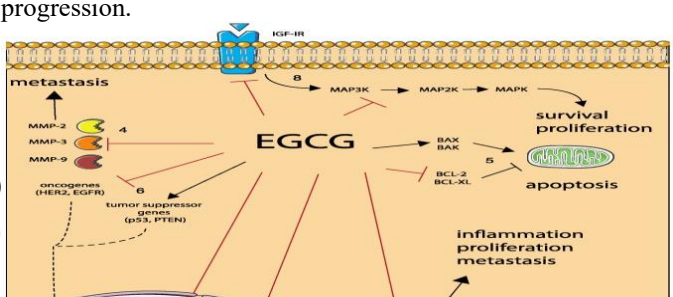
What Is EGCG?

Epigallocatechin-3-gallate, better known as EGCG, is a compound found in many natural foods. From a scientific standpoint, it's a polyphenol, which is a classification of plant-based organic chemicals distinguished by a large number of phenol structural groups. Polyphenols, including EGCG, occur naturally in a large number of plant foods like berries, fruits, seeds, and tea leaves. These compounds have been linked to a number of health benefits. They seem to work similarly to antioxidants within the body, decreasing inflammation and boosting energy.

EGCG is what's known as a water-soluble catechin, a particular type of flavonoid most often found in tea, cocoa, and berries. While catechins are widely accepted to be some of the most effective of the polyphenols, one member of this group stands out – EGCG. In fact, EGCG is known as the most potent antioxidant catechin. Berries and tea may be part of your regular diet, but a supplement is one of the best ways to ensure your body has access to this powerful compound.

In fact, though you may have berries at breakfast and tea with lunch, it's unlikely that such small amounts are able to help you get the full benefits of EGCG. Investing in a supplement ensures your body gets access to this full spectrum of benefits this compound has to offer. (6)

EGCG is involved in numerous signaling pathways and biological mechanisms related with cancer development and progression.



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Cancer oncogenes Human epidermal growth factor receptor 2 (HER2) and Epidermal growth factor receptor (EGFR); Inhibition of NF-κB and subsequent events of cell inflammation, proliferation, metastasis and angiogenesis; and Anti-proliferative activity by inhibition of Mitogen-activated protein kinases (MAPK) pathway and Insulin-like growth factor I receptor (IGFIR). (7)

EGCG Dosage

While researchers have been studying the potential health benefits and the various effects of EGCG for a few decades, this compound has continuously shown varied effects on human research subjects. Scientists believe this inconsistency may be caused by the tendency of the compound to degrade when exposed to oxygen, which leads to an ever-changing amount that actually reaches the digestive tract. Adding to the issue is the fact that many people aren't able to absorb EGCG efficiently through the digestive tract itself.

Currently, approved research lists the following safe dosage values:

- 704/day in liquid beverage form
- 338 mg/day as a concentrated solid dose

Because of the difficulty of absorption, it's important to take EGCG supplements from a reputable pharmacy that understands the best dosing for your unique body.

The Aim of the Work:

This Study aimed to evaluate the effect of Green Tea (Epigallocatechin-3-Gallate) on renal cell carcinoma in mice. A significant distinction between normal healthy cells and tumor cells is that the latter often circumvent the apoptosis process, allowing uncontrolled proliferation. Thus, inducing apoptosis would be an effective means of treatment. In RCC, the expression of tissue factor pathway inhibitor-2 (TFPI-2) is inversely related to the aggressiveness of these cells (Gu B., Ding Q., Xia G,……., 2009). (8)

Therefore, higher concentrations of TFPI-2 would decrease the malignancy of these cells and most likely induce apoptosis. Epigallocatechin-3-gallate (EGCG), an active and major constituent of green tea (Camellia sinensis), displays anti-tumor properties in several cancers, including RCC (9,10,11,12,13,14,15) and inhibits tumor growth and invasiveness in RCC by upregulating expression of TFPI-2 through inhibition of DNA methyltransferase (DNMT) activity.(8)

A recent paper indicates that EGCG may play a preventive role in the development of RCC. (16) This study evaluated the effect of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), EGCG, and a combination of both on a TRAIL-resistant RCC cell line, 786-O. The data demonstrate that EGCG alone provided a significant reduction in cell viability, but co-treatment with TRAIL provided a marked reduction in cell viability greater than that of EGCG or TRAIL alone by downregulating c-FLIP, MCl-1, and BCl-2.

Another study reported that EGCG induces apoptosis, inhibiting the proliferation and migratory potential of RCC cell lines by downregulating the expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9). (17) However, this study did not determine how the expression levels and activity of these metalloproteinases are regulated by EGCG. It is clear through multiple, independent experiments that EGCG has proven an extremely viable treatment in vitro. A few methods to utilize EGCG arise from the data previously presented. One example was an extensive epidemiological study which reported an inverse correlation between green tea consumption and overall RCC tumor burden. (18)

Another approach might use EGCG in supplement with TKI or mTOR inhibitors to see if the combination particularly sensitizes the tumor cells as compared to TKI or mTOR inhibitor alone. (19,20, 21, 22)

A study by Sato et al., suggests that the restoration of connexin 32 (Cx32) gene, a tumor suppressor, by EGCG pretreatment enhanced the chemical sensitivity of vinblastine via the inactivation of Src and the activation of the c-Jun NH2-terminal kinase (JNK) in RCC cells. (23) Overall, these studies suggest that EGCG could be used as both a preventative and therapeutic approach for renal cellcarcinoma.

Methods of preparation of nanoparticles

Nanomaterials and Nanotechnologies attract tremendous attention in recent researches. New physical properties and new technologies both in sample preparation and device fabrication evoke on account of the development of nanoscience. Various research fields including physics, chemists, material scientists, and engineers of mechanical and electrical are involved in this research. Synthesis of nanomaterials that are synonyms to quantum confined atom is an important milestone in the pursuit. Materials scientists and engineers have made significant developments in the improvement of methods of synthesis of nanomaterial solids. (Namita Rajput, 2015)

Nanoparticles are generally defined as particulate matter with at least one dimension that is less than 100 nm. This definition puts them in a similar size domain as that of ultrafine particles (air borne particulates) and places them as a sub-set of colloidal particles (SCENIHR 2005)

Nanoparticle structure:

Although it is often tempting to consider nanoparticles as simple molecules, they are in fact complex mixtures. Even in the simplest cases one must consider the interactions of at least two different aspects of the material. Whilst they may absorb light like a dye and appear to dissolve like any other small

molecule, their actual behaviour is often subtly different and is usually the result of the different components of the material. Any nanoparticle will have an exceptionally high surface area to volume ratio; this is one of the reasons for some of their unusual properties. However, this high surface area also means that the surface of any given nanoparticle is an important component of the material. So even the simplest nanoparticle will have a surface chemistry, which is distinctly different from that of the core material. In the case of silica the material will have a core structure of SiO2, but the surface would have a chemistry more comparable to a formula Si(O)(2−x)(OH)(2x) (Paparazzo 1992).

Preparation of nanoparticles:

There are two main methods for the preparation of nanoparticles: top down and bottom up. The former related to the preparation of nanoparticles by "cutting" larger pieces of a material until only a nanoparticle remains. This is commonly achieved using lithographic techniques or etching; however grinding in a ball mill can also be used in some cases. The more convenient method for producing nanoparticles on a commercial scale is to use a bottom-up approach where a nanoparticle is "grown" from simple molecules. The size of the nanoparticle may be controlled in a number of ways such as limiting the concentration, functionalizing the surface of the particle or using a micelle to template the growth. The bottom-up approach relies on the principle of supersaturation to control particle size.

In the early stages of the reaction the concentration of the final material rises rapidly, but no precipitation occurs until the saturation limit is reached (shown by the dotted line). If the reaction is proceeding fast enough the saturation limit may be exceeded before material begins to precipitate from solution. These initial particles will then act as seeds for the final particles and, in an ideal system, any further production of the final material will occur at the particle surface resulting in particle growth. This explanation is very simple and the reality may be much more complex (Christian and O'Brien 2005), but this principle is a defining factor in all bottom -up approaches. The general differences come from the medium in which the reaction is conducted, and this can play a key role in defining the final properties and surface chemistry of the particle. There is insufficient space here to consider all the permutations; however we will discuss some of the common methods employed in preparing the major classes of nanomaterials.

Nano mechanism:

(Applied Techniques)
Organic + inorganic solvent
Nano particles properties:

1. Easy to be absorbed (The smaller the size of the substance, the easier it is that it penetrates and enters the cell)
2. The results that supply by Nano are more effective.
3. Using a small size of the substance reduces toxicity and thus reduces side effects.

Procedure:

1. Use a water balance.
2. Keep balance at zero staging on the paper we weight on it.
3. The way to adjust the water balance is by centering the water point.
4. We use magnetic stirrer to adjust the temperature and the number of turns.
5. To convert matter into Nano, it must be dissolved so, dissolve each substance separately and then add them together.
6. The natural product will dissolve and remain in its size, but when you add PVA and leave them for an hour boiling, it begins to get smaller (due to evaporation of alcohol and then will reduce to the half of its original one) and transformed into Nano due to volatilization of alcohol.
7. Rolling speed and temperature depending on the solubility of substance

PVA "Polyvinyl Alcohol" :

- Water soluble in distilled water
- Dissolve in magnetic stirrer

1g PVA+ 10ml ethyl alcohol
1g natural product + 10ml distilled water

- PIGA the same as PVA concert it to Nano.

Mechanism:

1. Dissolve natural product in organic solvent (ethyl alcohol).
2. Weight by weight (PVA and natural product are the same weight).
3. Mix organic + inorganic together
4. The more the substance is small, the easy to enter the cell.
5. Finally, taken in tube (falcon 50) for examination under electron microscope.

Material and Methods:

- Experimental animals are divided into

Group I " control"

The 2 mice didn't receive any chemicals or treatment

Group II

The 6 mice were injected with cancer cells withdrawn from anther mouse which have solid tumor. Each mouse was injected with a 0.2 ml of cancer cells sample " Ehrlich solid carcinoma "subcutaneously.
The mice were left for (15) days before Necropsy & Samples Collection.

- **The Necropsy technique is as following:**

1. The mouse should first be examined externally and any obvious abnormalities should be noted and it should be anesthetized with chloroform.
2. Then placing the animal on its back on disposable absorbent paper the skin and abdominal muscles should be picked up using forceps and then carefully cut from the xyphoid cartilage along both flanks, completing the cut in front of the pelvic opening
3. The skin and muscle layers can then be placed abdominal side-down on fiber-free blotting paper
4. Next a cut should be made either side of the sternum to open up the thoracic cavity. Take care not to damage the heart and any other structures inside the cavity by holding the end of the xiphoid cartilage and cutting the thoracic tissue away close to the underside of the sternum
5. Holding onto the diaphragm with forceps, cut it away from the inside surface of the ribcage and cut the esophagus and associated tissue connections to the thorax, releasing the liver from the diaphragm and thoracic organs.
6. Once the diaphragm is free from the ribcage, push the abdominal viscera over to one side to access the blood vessels and tissue which secure the liver to the dorsal body wall. Slide the scissors carefully underneath the liver/stomach and spleen and over the top of the kidneys to cut free the upper portion of the viscera.
7. The intestines and associated organs can be removed from the carcass by cutting the terminal colon as close to the pelvis as possible.
8. Blood samples is collected by cutting renal vein. (24,25,26)

Laboratory tests performed on blood samples:

Alanine aminotransferase (ALT/GPT)

The enzyme alanine aminotransferase ALT is widely distributed with high concentrations in the liver and to a lesser extent in kidneys, heart, skeletal muscles, pancreas and lungs. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, liver carcinoma and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although both serum aspartate aminotransferase AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity (27,28,29).

ALT Procedure:

Assay conditions:
Analyzer: Spectrophotometer
Wavelength :320 nm
Cuvette :1cm light path
Temperature :30 -37C

1. Bring the working reagent and instrument to reaction temperature
2. Pipette into a cuvette:

Reaction temperature	**37 C**	**30 C**
Working reagent	1.0 ml	1.0 ml
Sample	50 µl	100 µl

3. Mix and insert the cuvette into photometer. Start the stop watch.
4. After 1 minute record initial absorbance and at 1 minute intervals thereafter for 3 minute.
5. Calculate the difference between consecutive absorbance and the average absorbance difference per minute
6. The ALT concentration in the sample is calculated using the following general formula

$$\Delta A/\text{min} * \frac{Vt * 10^6}{\varepsilon * I * VS}$$

ε: molar absorbance is 6300
I: light path is 1 cm
Vt: the total reaction volume is 1.05 at 37C
Vs the sample volume is 0.05 at 37C.

Aspartate aminotransferase (AST/GOT)

The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues such as myocardial infarction, viral hepatitis and muscular dystrophy. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the sera of patients with coronary and hepatobiliary diseases (27,28,29).

AST Procedure:

Assay conditions:
Analyzer: Spectrophotometer
Wavelength :320 nm
Cuvette :1cm light path
Temperature:30 -37C

1. Bring the working reagent and instrument to reaction temperature.
2. Pipette into a cuvette:

Reaction temperature	**37**	**30**
Working reagent	1.0 ml	1.0 ml
Sample	50 µl	100 µl

3. Mix and insert the cuvette into photometer. Start the stop watch.
4. After 1 minute record initial absorbance and at 1minute intervals thereafter for 3minute.
5. Calculate the difference between consecutive absorbance and the average absorbance difference per minute
6. The ALT concentration in the sample is calculated using the following general formula

$$\Delta A/\text{min} * \frac{Vt * 10^6}{\varepsilon * I * VS}$$

ε: molar absorbance is 6300

I: light path is 1 cm
Vt: the total reaction volume is 1.05 at 37C
Vs the sample volume is 0.05 at 37C.

Albumin is a carbohydrate-free protein, representing 55 – 65% of the total plasma proteins. It is synthesized in the liver and is noted for its ability of configuration changes. It maintains the plasma colloidal osmotic pressure, transports and stores a wide variety of ligands and serves as a source of endogenous amino acids. Albumin binds and solubilizes a variety of compounds amongst which are bilirubin, calcium, and long-chain fatty acids. Albumin also binds toxic heavy metal ions and many drugs, which is why a decrease in albumin in the blood can have important pharmacokinetic consequences **(30)**

Albumin test procedure:

Assay conditions: Colorimetric measuring by spectrophotometer
Wavelength:630nm
Cuvette:1cm light path
Temperature:15-25C/37C

1. Adjust the instrument to zero with distilled
2. Pipette into a cuvette

	Blank	Standard	Sample
R (ml)	1.0	1.0	1.0
Standard µl	…..	5	……
Sample µl	……	…..	5

3. Mix and incubate for 5 minutes at 37C or 10 minutes at 15-25C
4. Read the absorbance (A) of the sample and standard against the blank. the color is stable 1 hour at room temperature

1. Calculation:

$$\frac{(A)\text{sample}-(A)\text{blank}}{(A)\text{standard}-(A)\text{blank}} * 5 \text{ (Standard conc)}$$

The results of blood samples test

Test	Group I "control"	Group II "Ehrlich"
ALT	45	80
AST	60	150
Alb	3.5	2

Experimental Animals Pictures:

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