



## Natural Products: An Emerging Therapeutic Agents For Treatment of Cancer

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### ABSTRACT

Cancer is being a life threatening disease which can prove to be fatal for an infected patient if proper care or treatment is not taken in early stages. The cases of cancer are increasing day by day with the increase in the number of carcinogen. The natural therapy is required to be adopted to reduce the toxicity to other cells associated with available allopathic treatment. This review article mainly focuses on Natural products having various positive effects on the cancer cells and is very useful in the treatment of various type of the cancer and in the death of various carcinogens. It has been proved that the natural products show the reduction of growth of the cancer cells and other cytotoxic effect. Many of available drugs having the capability to reduce the growth of cancer cells by 90% but having serious side effects to other cells and sometimes it may leads to serious complications. Similarly many natural products have the ability of killing cancerous cells by 90% having minimal or no side effects. The natural products such as beta-glucan, cat's claw, vitamin C, mushroom extracts and various herbs, etc are useful in the treatment of cancer and cost effective. When the proper combination of such natural products is given it shows more potency to kill or inhibit growth of cancer cells and increase patient compliance by reducing treatment of cost minimal side effects.

**Keywords:** Cancer, Carcinogen, Natural Products, Cytotoxic Effect, Cat's Claw.

### INTRODUCTION

The disease cancer is increasing at an enormous rate and racing to the population rate. <sup>[1]</sup>The main objective of this review article is to understand the effect of the natural products on the cancer cells by reduction in cell growth or causing cell death and to acquire the knowledge if they are more active than synthetic therapy. The Use of natural therapy for the treatment of cancer which has very low chances of side effects on the host cells of the human body. Various natural products have ability to eradicate cancer and can be used, which are easily available in a large quantity and economical involving in unregulating cell growth. In cancer, cells divide, rapidly and uncontrollably, forming malignant tumors, and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous, Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans. Determining what causes cancer is complex. Many things are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, obesity, and environmental pollutants. These can directly damage genes or combine with existing genetic faults within cells to cause the disease. Approximately five to ten percent of cancers are entirely hereditary. <sup>[2,3,4]</sup>

Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. Cancer cells differs in four ways from the normal cells. <sup>[2,4]</sup>

1. Uncontrolled proliferation
2. Dedifferentiation and loss of function
3. Invasiveness
4. Metastasis

Cancer occurs through changes in the DNA sequence of our genome. Thus changes in the codon sequence keep in progress as our body comes in contact with UV radiations which can cause mutation. Cancer is mainly caused through environmental causes like the carcinogens such as nitrogen mustard, benzyl chloride, etc. sometimes it is caused by the carelessness and addiction of the people like addiction of chewing tobacco, smoking, etc and in few cases hereditary. The environmental causes are way more in the number than the hereditary. A mutation in signaling of the cell can send error-causing signals to nearby cells which may cause cells to become neoplastic, causing them to migrate and disrupt more healthy cells. It may also cause the cell to become immortal causing them to disrupt healthy cells forever. Thus cancer can also be defined as continuous dividing of the cells. Normal cells during division they pass through various phases of cell division. During this they produce an enzyme called telomerase which replaces the telomere. The cells stop producing the telomerase enzyme but the cancerous cells keep on producing this enzyme and do not stop dividing. Normal cells do depend on their DNA damage and cannot survive whereas cancer cells are independent of DNA damage and thus they live further and life span is more which creates tumors and mass. <sup>[5,6,7,8,9,10,11]</sup> Various class of anticancer drugs are being used but they have severe adverse effect such as alkylating agents such as Cisplatin is a good anticancer drug but it has an adverse effect which leads to deafness. Antimetabolites too have a large adverse effects such as mucositis, thrombocytopenia, cirrhosis, etc. <sup>[2,3]</sup> so if the natural products are being used that would be much better as the side effects would be less and if in any rural area is there then it can taken as raw as the natural activity would mostly depend on the active constituent present in that

particular plant which we are going to use for the treatment of cancer and some natural derivatives also helps in the prevention of cancer as in some cases we don't know the etiology of the cancer because in many times the environment causes are being seen so these chemical constituents will help in prevention of development of any cancer cells or preventing the normal cells from getting infected. Almost 31 natural herbs and plants or natural products are being used for the treatment of cancer which is spinach, Astragalus, beta-glucan, Baikal skullcap, calcium D-glucarate, cat's claw, cesium, chlorophyll, Chrysin, cordyceps, coriolus mushroom, etc<sup>[12,13,14,15,16,17]</sup>.

#### Effect of Cancer on human body<sup>[2,3]</sup>

Infection due to cancer causes two genetic changes. Out of which one is the activation of proto-oncogenes to oncogenes due to which the control over cell division, apoptosis and differentiation is lost. Another genetic change which occur is inactivation of tumor suppressor genes due to which the ability to suppress malignant change is lost. Thus due to formation of such cells the functions of the normal cells gets lost. The two types of tumors are being seen- benign tumor and malignant tumor.

- **Benign tumors** causes destroying of hypothalamus, atrial myxoma blocking the flow of blood through the heart, meningiomas compressing the brain, colloid cysts blocking the flow of cerebrospinal fluid, too much of tumor causes a lot of pain, GI problems which causes

bleeding, potassium wasting, obstruction, hormone production, torsion and infarction, etc

- **Malignant tumors** are of various types depending on the site where they are forming and once they are removed they are seen again but at different site. If any of that cancer is not treated successfully it will eventually kill the patient, causing edema, and different type of this tumors show different effect on the body.

#### Natural products and their effect on the cancer cells

Generally three methods are being used for the treatment of cancer which are surgery, radiation and chemotherapy. In the chemotherapy generally the use of various drugs is done such as alkylating agents, folic acid analogs, Antimetabolites, natural derivatives, etc. now out of all these class of anticancer drugs, the most interesting study is done on natural derivatives. Various herbs, plants, natural products such as spinach, Astragalus, beta-glucan, Baikal skullcap, calcium D-glucarate, cat's claw, cesium, chlorophyll, Chrysin, cordyceps, coriolus mushroom, etc. are being used in the treatment of the cancer. The mechanism and effect of the natural products vary and so their efficacy. Various natural products are showing the following effect. It is estimated that there are 31 herbs and natural products known used in treatment of cancer.<sup>[15,19]</sup> Some of the natural products and there effects are shown in Table: 1 as below:

**Table 1:** Effect of Different natural product on various types<sup>[15, 19, 20, 21]</sup>

Sr. No.	Natural Products	Cancer Type	Effects
1.	Astragalus saponins	Colon cancer	Inhibit cell proliferation through accumulation in S phase and G-2/M arrest, it reduces the tumor volume as well as pro-apoptotic and anti-proliferative effects
2.	Beta-glucan	Breast cancer	Obtained from mushroom and yeast and in wheat too, it helps in raising the activity of the macrophage and NK cells in the body which helps in fighting against the cancer cells. It also inhibits the hepatic metastases which inhibit further growth of tumors and thus enhance long term survival of the patient.
3.	Baikal skullcap	Leukemia, prostate, lung cancer	Prevention of proliferation of malignant tumors, angiogenesis and causes apoptosis.
4.	Calcium D-glucarate		It has anticarcinogenic properties in addition to glucuronidation. It generally conjugates with the carcinogen or cancer cells and then they are excreted through the biliary tract which is helpful during the treatment of cancer.
5.	Cat's claw	Leukemia	It is helpful in treating feline leukemia virus. It is used to stimulate the immune system. The oxindole alkaloids especially isopterodine and pterodine stimulate phagocytosis.
6.	Cesium	Leukemia	Help in disappearance of pain in all patients within one to three days after initiation of the treatment. It changes the pH from acidic to alkaline due to which the pain sensation disappears.
7.	Chrysin	Breast cancer	Aromatase inhibiting action which is helpful for women in breast cancer
8.	Squalamine	Human ovarian cancer, lung cancer.	Blocks blood vessels growth in tumors, it blocks the growth factor due to which the growth of cancer cells either stops or reduces.
9.	Fish oils	Breast cancer	Immune system stimulation, tumor cell toxicity, alteration of cell membrane.
10.	Vitamin C	Lung cancer, melanoma skin cancer	It helps in killing the cancerous cells and also in inhibition of cancerous cell growth.

Thus many such natural products such as chlorophyll, honey, etc are useful in the treatment of cancer by cytotoxic effect and inhibition of cell growth. The effects of the natural products can be known and studied by performing the following experiment where some mushroom extracts and vitamin C is being used.

#### Official Bioassay of Determine the Cell Growth Inhibition Activity of Natural Products:<sup>[22,23]</sup>

Various experiments regarding the effects of natural products can be done in many ways but to show the effect of natural products as they decrease the growth of the cancerous cells. Experiment for determining the inhibition

of cancer cell growth. The following experiment will show the effect of natural products to inhibit of cell growth.

Certain mushroom extract act on the bladder cancer and reduces its growth. So Bladder cancer T24 cells are being taken from the patient infected with transitional cell carcinoma. These cells were maintained in McCoy's 5a medium which consists of 10% fetal bovine serum, penicillin (100u/ml) and streptomycin (100microgram/ml). The culture medium is changed after every 3-4 days and the passage of cells was performed weekly. For experiments these cells were seeded in six-well culture plates at an initial cell density of  $1 \times 10^5$  cells/ml. T24 cells were cultured for 72 hours with varying concentration of different natural products. Cell morphology, appearance, cell number and viability can be checked using trypan blue exclusion test and Alamar Blue cell viability test.

#### Trypan Blue Exclusion Test:<sup>[22,23]</sup>

In trypan blue exclusion test, trypan blue dye is being used in the infected cancerous cells, the viable cells uptake the dye and which shows a clear vision of the viable cells and thus they can be differentiated. Thus by counting the viable cells or general movement in the vision of the cells, reading can be recorded.

#### Alamar Blue cell viability test:<sup>[22,23]</sup>

In Alamar Blue cell viability test, the alamar blue dye is used for the cell viability test. 96-well plate is being used in this test. You can examine 50 cells per well. Serial dilution of alamar blue is done at 1/10 volume in black,

clear bottom 96-well plates and fluorescence was measured after 40 minutes and 18 hours. The effect of the natural products depends on the intensity of fluorescent light emerging from the 96-well plate. It works on the mechanism that it contains resazurin, a non fluorescent indicator dye, is converted into highly red-fluorescent resorufin via redox reactions in metabolically active cells. Thus it indicates the number of the active and living cells. The following natural products are being tested by the above experiment and the study of their effect on the cancerous cells was being observed.

- 1) Active fraction of maitake mushroom
- 2) Extract of meshimakobu mushroom
- 3) Extract of yeast cell wall
- 4) Extract of Agaricus blazei Murill mushroom
- 5) Arabinoxylan from rice bran
- 6) Mixed powder of Agaricus blazei Murill mushroom and shark cartilage
- 7) Mixed powder of three kinds of mushrooms and three herbs
- 8) Mycelial extract of several mushrooms
- 9) Vitamin C

These natural products are being tested one after another on the cancerous cells and the concentration of them is varied too and the following effect is shown by some of the natural products on the cancerous cells. Sometimes to obtain better result in the effect on the cancer cells the combination of the natural products can be given. The results show in the following table-2.

**Table 2:** Results of various Natural Products with their Different Effective Concentration and Effects<sup>[24]</sup>

Sr. No.	Natural product	Effective Concentration	Effect
1	Active fraction of maitake mushroom	60 µg/ml	No effect
		120 µg/ml	25% reduction in cell growth
		≥ 240 µg/ml	≥ 90% reduction in cell growth
2	Extract of meshimakobu mushroom	20 µg/ml	No effect
		50 µg/ml	30% reduction in cell growth
		100 µg/ml	80% reduction in cell growth
3	Vitamin C	≥ 200 µg/ml	≥ 90% reduction in cell growth
		100 µM	No effect
		200 µM	No effect
		300 µM	25% reduction in cell growth
		500 µM	50% reduction in cell growth
4	Extract of yeast cell wall	700 µM	90% reduction in cell growth
		1000 µM	No cell growth
		20 µg/ml	≥ 90% cell death

All the above mentioned natural products showed reduction in the growth of cancerous cell. The other natural products such as Extract of Agaricus blazei Murill mushroom, Arabinoxylan from rice bran, Mixed powder of Agaricus blazei Murill mushroom and shark cartilage, Mixed powder of three kinds of mushrooms and three herbs show very little effect even when they are used in high concentration.

#### Etiology<sup>[25]</sup>

Cause that is not inherited genetically, not merely pollution. 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. Substances that

cause DNA mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens. Many mutagens are also carcinogens, but some carcinogens are not mutagens. Alcohol is an example of a chemical carcinogen that is not a mutagen. Some hormones play a role in the development of cancer by promoting cell proliferation. 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. Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer is generally not a transmissible disease.

#### Pathogenesis<sup>[25]</sup>

Cancer occurs when a single progenitor cell accumulates mutations and other changes in the DNA, histones, and

other biochemical compounds that make up the cell's genome. The cell genome controls the structure of the cell's biochemical components, the biochemical reactions that occur within the cell, and the biological interactions of that cell with other cells. Certain combinations of mutations in the given progenitor cell ultimately result in that cell (also called a cancer stem cell) displaying a number of abnormal, malignant cellular properties that, when taken together, are considered characteristic of cancer, including:

- The ability to continue to divide perpetually, producing an exponentially (or near-exponentially) increasing number of new malignant cancerous "daughter cells" (uncontrolled mitosis);
- The ability to penetrate normal body surfaces and barriers, and to bore into or through nearby body structures and tissues (local invasiveness);
- The ability to spread to other sites within the body (metastasize) by penetrating or entering into the lymphatic vessels (regional metastasis) and/or the blood vessels (distant metastasis).

If this process of continuous growth, local invasion, and regional and distant metastasis is not halted via a combination of stimulation of immunological defenses and medical treatment interventions, the end result is that the host suffers a continuously increasing burden of tumor cells throughout the body. Eventually, the tumor burden increasingly interferes with normal biochemical functions carried out by the host's organs, and death ultimately ensues.

Mycelial extract of several mushrooms but when they were used with another natural product such as Vitamin C, it showed better effect and it helped in killing the cancerous cells (cytotoxic effect) as shown in Table-3

**Table 3:** Effect Vitamin-C and Active Extracted fractions of several Mushrooms with their effects <sup>[26]</sup>

Vitamin- C	Active fraction of Maitake Mushroom	Extract of Meshimakobu Mushroom	Effect
200 µM	30 µg/ml	-	> 90% cell death
200 µM	-	20 µg/ml	> 90% cell death

Thus the above experiment explains the property of natural products of inhibiting cell growth and cytotoxic effect by causing the cell death. Some of the natural products show the cytotoxic effect whereas some are useful in inhibition of the growth of the cancerous cells.

Some of the above natural products such as mushroom extract and vitamin c show effects on the cancerous cells. Active fraction of Maitake mushroom and Extract of Meshimakobu mushroom when inserted in the cancerous cells shows their effect. It was observed that the intensity of fluorescent in Alamar blue dye test was decreasing with the increase in the concentration of the natural products which shows that there was depletion in the growth of the cancer cells. They mainly cause the inhibition of the growth of the cancer cells. As the concentration of these natural products increases, the effect increases and there in reduction in the growth of cancerous cells to a great extent. Both of them were required in amount of 240µg/ml and 200µg/ml

respectively for causing 90% reduction in growth of cancer cell but when they were used in combination with vitamin C they were used 30 µg / ml and 20 µg / ml respectively to cause 90% cell death. Vitamin C itself doesn't give cytotoxic effect but in combination with active fraction of Maitake mushroom and extract of Meshimakobu mushroom it gives the cytotoxic effect. Therefore when used in proper combination of natural products for the treatment of cancer, it is proved to be more efficient and potency becomes high whereas extract of yeast cell wall directly gives the cytotoxic effect on the cancerous cells. Thus these natural products can be very useful in the treatment of cancer as the side effect is less. <sup>[26]</sup>

From the experiment which was being performed, it was concluded that natural products do have some effect on the cancer cells in the body of an infected person. From the result it was shown that natural products do show two main promising effects on the cancer cells

- A. They inhibit the proliferation of the cancer cells which results into the inhibition of the growth of the cancer cells.
- B. They show cytotoxic effect due to which the activity of macrophage and NK cells increases which helps in killing the cancerous cells from the body. <sup>[27,28]</sup>

## CONCLUSIONS

Natural products are emerging novel approach for the treatment of cancer and enlarge the fields of pharmaceutical researchers. It can be predicted that the exercise of natural product in the treatment of cancer is favorable, due to other available treatment in the market employed with high treatment cost and chances of severe side effects by acting on other than cancerous cells which causes some serious complications comparing to natural product. Even use of natural product in treatment of cancer is cost effective because it is extensively available so it is affordable by patients and hence increases patient's compliance.

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