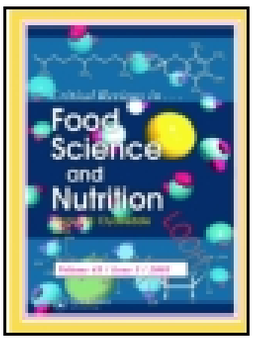

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Anticancer potential of dietary vitamin D and ascorbic acid: A review

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Abstract:

Cancers have been the leading cause of death worldwide and poor diet and physical inactivity are major risk factors in cancer-related deaths. Micronutrients such as vitamins and minerals appear to have preventive properties against cancer. One important mechanism by which dietary changes can exert preventive effects on cancer is via modulation of micronutrient concentrations in target tissues. Many of these micronutrients are available in the form of dietary supplements, and the intake of these supplements is prevalent in various parts of the world. However in most cases it is not known which micronutrient (or combination of micronutrients) is best when it comes to lowering the risk of cancer. The present review illustrates the effect of vitamin D and ascorbic acid intake on preventing cancer.

Keywords: Cancer; Dietary supplementation; Vitamin D; Ascorbic acid; anti-neoplastic

Introduction:

Cancer is currently one of the most important public health issues. World Health Organization (WHO) reported that 14% of global deaths resulted from malignant neoplasm, and the incidence is expected to be 18% in 2030 (Kwan et al, 2015). Cancers are not only restricted to human beings; domestic animals and pets are also at an equivalent risk. The frequency of neoplasia in different species varies tremendously. Dogs are by far the most frequently affected domestic species, with a prevalence ~3 times that in women, followed by cats and other pets (Chaudhary et al., 2014; Sunil Kumar and Kataria, 2013). It is nearly impossible to prove what caused a cancer in any individual, because most cancers have multiple causes. Over 30% of cancers are potentially avoidable by reducing key risk factors, like tobacco use and obesity, which respectively cause about 22% and 10% of cancer deaths (Cancer fact sheet, WHO, 2014). In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C, and human papillomavirus (Cancer fact sheet, WHO, 2014). Approximately 5ï 10% of cancers are due to genetic defects inherited from a person's parents (Word cancer report, 2014). Apart from genetic causes which were predominant in the earlier times, environmental causes and changed lifestyle have increased the incidence of cancer these days (Anand et al., 2008). Dietary factors are among the most important environmental factors implicated in most of the cancers. It has been estimated that around 10-70% of all cancers are attributable to diet and that up to 90% of colorectal cancer may be avoidable through alterations in the diet and life style (Bishayee et al., 2015). Although treatments like surgery, chemotherapy, radiation therapy are available; cancer is relatively incurable till date and may even relapse after treatment.

Early detection of neoplasmic growths can save lives, but cancer is often detected as late end point of carcinogenesis, so, its early diagnosis is very poor (Sunil Kumar, et al., 2013). Hence, best defense against cancer is prevention. Certain types of cancer, caused by viruses can be prevented by prophylaxis, however, the efficacy of anti-tumor vaccines is plausible. The evidence linking poor nutrition, overweight/obesity, and physical inactivity as risk factors for cancer continues to develop and a healthy diet is one of the most important lifestyle changes that a person can make to reduce his/her risk of cancer (Kushi et al., 2012; Beaglehole et al., 2011). WHO has graded the strength of the evidence for these lifestyle risk factors, in relation to a range of cancers, and estimated that around 1 million new cases could be prevented in the USA, UK, China and Brazil alone, by merely correcting lifestyle behavior. Reducing the risk of certain cancers may be possible through the usage of dietary supplements, which could be used alone or in combination with conventional chemotherapeutic agents, in order to prevent new cases of cancer (Pelucchi et al., 2009; Visioli et al., 2005).

Dietary supplements include vitamins, minerals, herbs, or products made from plants. They can also be made from animal parts, algae, seafood, yeasts, fungus, and many other food substances or extracts. They also include powdered amino acids, enzymes, energy bars, and liquid food supplements. Used properly, certain dietary supplements may help in reducing the risk of some diseases and discomfort caused by certain drugs or conditions. And most people can use dietary supplements safely within certain dosage guidelines.

A number of epidemiologic studies have investigated whether people with higher vitamin intakes or higher blood levels of certain vitamins have lower risks of specific cancers. The

cancers for which the most human data are available are colorectal, breast, prostate, and pancreatic cancer. Numerous epidemiologic studies have shown that higher intake or blood levels of vitamin D are associated with a reduced risk of colorectal cancer. Experimental evidence has also suggested a possible association between vitamin D and cancer risk. In studies of cancer cells and of tumors in mice, vitamin D has been found to have several roles that might slow or prevent the development of cancer, including promoting cellular differentiation, decreasing cancer cell growth/proliferation, stimulating cell death (apoptosis), and reducing tumor blood vessel formation (angiogenesis) (Ma et al., 2011; Gandini et al., 2011). Ascorbic acid (Vitamin C) exhibits antioxidant actions including the neutralization of free radicals (Sunil Kumar et al., 2010) which may impact cancer progression. At higher doses it may also act as a pro-oxidant as *in vitro* experiments have demonstrated cytotoxicity to cancer cells without similar effects on normal cells (Harris et al., 2014).

In this article, we critically review the effectiveness of dietary micronutrients especially vitamin D and ascorbic acid in reducing cancer risks. The anticancer mechanisms of these dietary vitamins are also discussed.

Vitamin D and its fortification in foods

Vitamin D is a group of fat-soluble micronutrients that also act as pro-hormone. It helps in deposition of calcium and phosphorus in bones and teeth. Vitamin D is synthesized within the body on exposure to sunlight. However, it can also be obtained from certain foods (Table 1). Dietary sources include a few foods that naturally contain vitamin D, such as fatty fish, fish liver oil, and eggs. However, most dietary vitamin D comes from foods fortified with vitamin D, such

as milk, juices, and breakfast cereals. The Food and Nutrition Board (FNB) at the Institute of Medicine of The National Academies (formerly National Academy of Sciences) has recommended daily intakes of vitamin D, assuming minimal sun exposure (Table 2). Vitamin D deficiency leads to rickets in children and osteomalacia in adults which are characterized by weak bones and cartilages. Vitamin D exists in two major forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is made naturally by plants, and vitamin D3 is synthesized by the body when exposed to sunlight. Both forms are converted to 25-hydroxycholecalciferol in the liver which is further modified to 1,25-dihydroxycholecalciferol (calcitriol), the active form of vitamin D in the body (Norman, 1998).

Excessive intake of any nutrient can cause toxic effects and vitamin D is no exception. Too much vitamin D can be harmful because it increases calcium levels, which can lead to calcinosis (the deposit of calcium salts in soft tissues, such as the kidneys, heart, or lungs) and hypercalcemia. The safe upper intake level of vitamin D for adults and children older than 8 years of age is 100 ε g per day (4000 IU per day). Toxicity from too much vitamin D is more likely to occur from high intakes of dietary supplements. Excessive sun exposure does not cause vitamin D toxicity. However, the Institute of Medicine (IOM), Food and Nutrition Board states that people should not try to increase vitamin D production by increasing their exposure to sunlight because this will also increase their risk of skin cancer (Otten et al., 2006).

Sun exposure is the major requisite for vitamin D synthesis. Despite plentiful sunshine, vitamin D deficiency prevails in tropical countries like India due to several socioeconomic and cultural constraints (Ritu and Gupta, 2014). Fortification of widely consumed staple foods with

vitamin D offers one of the simplest and most practical methods to combat its deficiencies for both poor and wealthy societies. General principles for the addition of essential nutrients to foods are given by the Codex Alimentarius Commission. The Codex definition of fortification is ñthe addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groupsò. The advantages of fortification include greater specificity of intervention and better dose adjustment. However, there are limitations of this strategy as well such as more cost, low compliance, self-prescription and increased risk of toxicity. Several foods may be fortified with vitamin D in tropical countries like India, e.g., milk, yogurt, infant formulas, ghee, soy milk, sugar, etc. Some of the vitamin D fortified foods are discussed as follows.

Vitamin D fortified Milk:

Milk and milk products are preferred vehicles to fortify with vitamin D all over the world. Prior to fortification of milk with this fat soluble vitamin, homogenization should be done to achieve a uniform distribution of added vitamin D throughout. India is the largest producer of milk in the world. Fortification of milk with vitamin D could aid in correcting vitamin D deficiency in Indian population. However, major hindrances to its proper use are milk adulteration and high prevalence of lactose intolerance in the tropical population (Ritu and Gupta, 2014).

Vitamin D cereal flour:

Bread made up of flour is one of the staple foods consumed all over the world. Various cereals may be used to make flours viz wheat, corn, millet, sorghum/milo, barley etc. Fortification of flour with vitamin D is a very viable fortification vehicle. Promising results have been obtained regarding technological feasibility and bioavailability of vitamin D in fortified wheat flour (Madsen et al., 2013; Mocanu et al., 2009; Natri et al., 2006). Notably, bread fortified with 5000 IU of vitamin D3 per daily serving consumed by sun-deprived nursing home residents in Romania demonstrated both the efficacy and safety of wheat flour fortification. Fortification of bread resulted in higher serum concentrations of vitamin D (>30 ng/mL) and significantly increased bone density (Mocanu et al., 2009).

Vitamin D fortified refined flour:

Refined wheat flour is widely used in bakeries for the manufacturing of cakes, pastries, biscuits, etc. Its fortification may have a significant contribution in the improvement of the vitamin D status of the general population. However, most of the vitamin losses occur during baking at temperatures over 200 °C. But the temperature inside the product is significantly lower, so the thermal degradation of vitamin D would be within an acceptable range (Ritu and Gupta, 2014).

Vitamin D fortified rice:

Rice is the most widely consumed staple food in the tropical world. Important fortification strategies in rice include hot & cold extrusion, coating and dusting (Alavi et al., 2008). In hot extrusion technique, dough made of rice flour, a fortificant mix and water is passed

through an extruder where it is cut into grain-like structures that resemble rice kernels. This process involves relatively high temperatures (70-110^{o} C). It results in fully or partially pre-cooked simulated rice kernels that have similar appearance (sheen and transparency) as regular rice kernels. Similar steps are followed in cold extrusion technique as well except that, in this case rice-shaped simulated kernels are produced by passing the dough through a simple pasta press at a temperature below 70^{0}C. Coating combines the fortificant mix with ingredients such as waxes and gums. The mixture is sprayed over the surface of grain kernels in several layers to form the rice-premix and then is blended with polished rice. Dusting involves sprinkling the polished rice grains with the powder form of the micronutrient premix (Alavi et al., 2008).

Vitamin D fortified cooking oils and spreads like butter and ghee:

Fats, margarine and cooking oils are attractive fortification vehicles for vitamin D all over the world owing its fat soluble nature (Calvo et al., 2013; Yang et al., 2013). Fortification of margarine with vitamin D is mandatory in Canada and optional in the USA (Calvo et al., 2013). Cooking oils are not fortified in the USA and Canada (Calvo et al., 2004). However frying consumables in vitamin D fortified fats may result in loss of about 50% of vitamin D (Lu et al., 2007) so cooking with very little oil is advisable, i.e., baking or broiling (oil-free) instead of frying (shallow or deep). However, overconsumption of these products may exacerbate other health issues related to metabolic syndrome.

Anti-cancer effect of vitamin D

Vitamin D not only regulates calcium and phosphate metabolism, but its antimitotic and cell differentiation effects have also been recently documented. It can inhibit cell-proliferation, neo-angiogenesis and metastatic potential in cancerous tissue. Vitamin D deficiency has also been associated with higher aggressiveness of tumor and poor prognosis. Although clinical studies showing benefit of vitamin D supplementation on cancer outcome are absent, clear recommendations are currently available for treatment of vitamin D deficiency during anti-cancer therapy. Owing to the high prevalence of vitamin D insufficiency in cancer patients and significant risks of its further decrease after antitumor therapy, it should become standard of care to examine 25- hydroxyl cholecalciferol serum levels and correct vitamin D insufficiency in cancer patients (Tomiska et al., 2015). Calcitriol, the active form of vitamin D, is known to inhibit the proliferation and invasiveness of many types of cancer cells, including breast, colon, pancreatic, prostate, and liver cancer cells. Several vitamin D analogues that are less calcemic but exhibit more potent anti-tumor activity have also been recommended as good candidates for anti-cancer therapy (Matsumoto et al., 2015).

Many studies have revealed a link between vitamin D and breast cancer as well. Women with breast cancer tend to have low levels of vitamin D in their body. Studies reveal that maintaining an optimal 25 hydroxy cholecalciferol status at diagnosis and during the 1-year follow-up period is important for improving breast cancer patient survival (Lim et al., 2015). Vitamin D receptors in breast tissue, upon binding with vitamin D triggers apoptosis in cancerous cells and prevent metastasis (Rose et al., 2013). However, the relationship between breast cancer and vitamin D is complex, not fully understood, and is still being studied (Wang et al., 2013). Some studies found that women with low levels of vitamin D are more likely to

develop breast cancer. A recent review of many studies found that post-menopausal women with low levels of vitamin D had a higher risk of getting breast cancer compared to post-menopausal women with high levels of vitamin D (Bauer et al., 2013). In a dose-response relationship study, it has been found that increased vitamin D levels in the body decreases breast cancer risk (Bauer et al., 2013).

However, most prevention studies on vitamin D and cancer have been observational. No studies have been undertaken for treating cancer patients with vitamin D. Some studies have shown that there is a link between vitamin D levels and recurrence of breast cancer, tumor size, and death from breast cancer. Studies indicate that women with breast cancer who had low vitamin D levels had a more than doubled risk of cancer relapse and an almost doubled risk of death compared to women with high vitamin D levels.

There is a growing amount of observational research that shows a protective anti-cancer effect of vitamin D (Rose et al., 2013; Goodwin et al., 2009). A study was conducted in USA on a large group of women who received calcium (1,000 mg) plus vitamin D (400 IU) supplement or a dummy pill for 7 years. After almost 5 years of supplementation, women who were in the calcium plus vitamin D group had an 18% lower risk of developing early stage breast cancer (Cauley et al., 2013). In a different experiment, a vitamin D (1,000 IU) plus calcium (1,400-1,500 mg) supplementation was found to reduce the incidence of any kind of cancer after 4 years of supplementation (Chlebowski et al., 2008)

Another study found that post-menopausal women with higher vitamin D levels had a 12% lower chance of developing breast cancer than post-menopausal women with low vitamin D

levels. However a threshold effect was observed with no additional decrease in breast cancer risk when the women had vitamin D levels above 35 ng/mL (Bauer et al., 2013).

Molecular mechanisms underlying anti-cancer effect of vitamin D

Calcitriol has emerged in recent years as a promising agent with anti-cancer properties (Krishnan et al., 2005; Moreno et al., 2005; Konety and Getzenberg, 2002; Miller, 1998; Feldman et al.,1995). It is an important regulator of calcium homeostasis and bone metabolism through its actions in intestine, bone, kidney and the parathyroid glands (Moreno et al., 2005). In addition to these classical actions, calcitriol also exerts anti-proliferative and pro-differentiating effects in a number of tumors and malignant cells raising the possibility of its use as an anti-cancer agent. The effects of this fat soluble vitamin are varied, appear to be cell-specific and result in growth arrest and stimulation of apoptosis (Fig. 1).

Effect of vitamin D on cell proliferation and apoptosis

Regulation of cell cycle is mediated by a complex network of interlinked regulators that govern cellular proliferation in an orchestral fashion. These regulators include proteins like cyclins and their association with enzymes known as cyclin dependent kinases (CDKs) and CDK inhibitors (CKIs). Tumor progression occurs when the rate of cell proliferation exceeds that of cell death. Cell death can occur either by necrosis (the result of tissue insult or injury), or active cell death (apoptosis), an energy-dependent process in which a distinct series of biochemical and molecular events lead to the death of cells by specific signals (Wyllie, 1987). The significance of

apoptosis in cancer research is emphasized by the recognition that many chemotherapeutic agents induce tumor regression through their ability to activate apoptosis.

Cell-cycle perturbation is central to calcitriol-mediated anti-proliferative activity in tumour cells. Vitamin D may exert growth inhibitory effects through repression of different key molecules involved in cell cycle regulation. Calcitriol treatment of human breast cancer cell line MCF-7 was found to repress c-Myc, a known proto-oncogene in the cell cycle regulatory machinery (Jensen et al., 2001). Calcitriol can also suppress expression of few oncogenes thereby increasing expression of their antagonists in the signaling pathway (Salehi-Tabar et al., 2012; Meyer et al., 2012; Washington et al., 2011). When ovarian cancer cells were treated with calcitriol, cell cycle was found to be arrested in G(1) phase through down-regulation of cyclin E/cyclin-dependent kinase 2 (Li et al., 2004). Collectively, these studies confirm that active form of vitamin D can suppress cell proliferation through inhibitory effects on several regulators in the network of cell cycle control machinery.

In addition to the anti-proliferative effects of vitamin D3, there is increasing evidence of its anti-tumour effects by regulating key mediators of apoptosis, such as repressing the expression of the anti-apoptotic proteins BCL2 and BCL-XL, or inducing the expression of pro-apoptotic proteins (such as BAX, BAK and BAD) (Deeb et al., 2007). Vitamin D treatment has also been found to induce p53 independent apoptosis in-vitro, It also increases levels of the pro-apoptotic protein, Bak (a member of the BCl-2 gene family), in many cell lines. In rat glioma cell lines, vitamin D treatment induced apoptosis via DNA fragmentation and upregulation of p53 genes (Baudet et al., 1996). In prostate cancer cell lines, vitamin D treatment inhibited cyclin-

dependent kinase 2 activity and induced G0/G1 cell cycle arrest (Bao et al., 2004). Therefore, several lines of evidence from in vitro studies support the role of vitamin D in promoting cell cycle arrest and promoting apoptosis in malignant transformed cells.

Effect of vitamin D on oxidative stress and angiogenesis

Tumor progression is often accompanied with changes in the normal physiology of the cells including production of free-radicals that may induce oxidative stress in-vivo. Oxidative stress can induce DNA damage and loss of DNA-repair ability (Valko et al., 2007). Vitamin D activates cellular signaling cascades that reduce thioredoxin and promote antioxidant responses, induce mRNA expression of superoxide dismutase and downregulates glutathione levels by increasing glucose-6-phosphate dehydrogenase expression (Fleet et al., 2012; Bao et al., 2008).

Neo-angiogenesis is a requisite for survival of cells in tumor micro-environment, as the growing tumor mass requires more oxygen supply. Cancer progression often induces hypoxia which in turn promotes hypoxia-inducible factor 1 (HIF-1)-dependent angiogenesis essential for tumor growth (Losso et al., 2005). Vitamin D3 has been found to inhibit cellular neo-angiogenesis in several cancer cell lines. Upon addition of vitamin D3 to the androgen-insensitive prostate cancer cell line, their proliferation both in normoxia and in hypoxic environments (that resemble those in cancer tissues) was found to decrease. Vitamin D3 has also been found to inhibit secretion of vascular endothelial growth factor (VEGF) in breast cancer cell line. Furthermore, vitamin D treatment downregulates endothelin 1 (ET-1) and glucose transporter 1 (Glut-1) that are essential for inducing angiogenesis. This molecular effect is

mediated via significant downregulation of HIF-1 transcription and translation (Ben-Shoshan et al., 2007).

Vitamin D receptor and cancer

Besides its other physiological roles, vitamin D acts in an intracrine or autocrine manner and stimulates the transcription of multiple genes involved in cellular differentiation via binding to its receptor VDR, a nuclear transcriptional factor which belongs to the super family of steroid/thyroid hormone receptors, located in the cell nucleus and expressed ubiquitously in most body tissues as well as on cancerous cells (Bertone-Johnson, 2009; Townsend et al., 2005).

It has been hypothesized that a less active VDR, could be associated with either an increased susceptibility to cancer risk or a more aggressive disease. A decrease in VDR protein expression, due to a functional impairment, may be influenced by the polymorphism in the VDR gene. Thus, polymorphisms in the VDR gene may be involved in the development and or progression of certain kinds of tumors (Pulito et al., 2015; Iqbal et al., 2015; Xu et al., 2003; Chan et al., 2000). It has been previously demonstrated that VDR expression is reduced in cancer cells (Lopes et al., 2010), which may be influenced by polymorphisms within the gene and increase breast cancer incidence (Tang et al., 2009).

Vitamin D catabolism and cancer

Calcitriol is synthesized from vitamin D in a highly regulated cascade. Firstly, vitamin D3 (cholecalciferol) is formed in the skin through the action of ultraviolet irradiation. Vitamin D3 then gets converted to 25-hydroxycholecalciferol [25(OH)D3] by liver mitochondrial and

microsomal enzyme (25-hydroxylases), encoded by the gene CYP27A1. 25(OH)D3 is then converted to 1Ű,25(OH)2D3 (calcitriol) in the kidney by mitochondrial 1Ű-hydroxylase (encoded by the gene CYP27B1). 24-hydroxylation of 25(OH)D3 and calcitriol by the cytochrome P_{450} enzyme 24-hydroxylase, to the metabolites 24,25(OH)2D3 and 1Ű,24,25(OH)2D3, respectively, is the rate-limiting step for 25(OH)D3 and 1Ű,25(OH)2D3 catabolism (Deeb et al., 2007).

Calcitriol functions in an autocrine and paracrine manner to modulate vitamin D function and signaling. It represses CYP27B1 while induces CYP24A1 to produce the less active vitamin D metabolites viz. 1Ű,24,25(OH)2D3 and 24,25(OH)2D3 (Brenza and DeLuca, 2000). 1Ű-OHase is expressed in tissues other than kidneys also such as colon, brain, placenta, pancreas, lymph nodes and skin (Zehnder et al., 2001), allowing non-renal synthesis of calcitriol. CYP27B1 (encoding 1Ű-hydroxylase) is found to be over-expressed in breast and prostate cancers (Townsend et al. 2005; Schwartz et al., 1998). Increased expression of CYP27B1 in cancer tissues could provide local synthesis of calcitriol. However, CYP24A1 (encoding 24-OHase) is upregulated in tumours, and may counteract calcitriolôs anti-proliferative activity, by increasing its catabolism.

Inhibition of CYP24A1 expression and activity is essential for cancer prevention. Chemical inhibitors with varying specificity for 24-OHase render tumour cells more sensitive to the action of calcitriol and its analogues (Parise et al. 2006; Peehl et al., 2002). This indicates that calcitriol catabolism could modulate tumour growth in some tissues, indicating the potential for the development of 24-OHase inhibitors as cancer preventative and/or anticancer therapeutic agents (Deeb et al., 2007).

The above mentioned findings support that vitamin D can effectively be used to prevent cancer. Various epidemiological studies indicate that inadequate levels of vitamin D are associated with an increased risk and poor prognosis of several types of cancer. Meta-analysis and cancer-prevention trials indicate that vitamin D supplementation can lower the incidence of many cancers including colorectal cancer, breast and prostate cancer. However, random studies need to be undertaken to optimize effective dose of vitamin D to prevent cancer.

Ascorbic acid (Vitamin C)

The consumption of fruit and vegetables exerts a preventive effect towards cancer and in recent years natural dietary agents have attracted great attention in the scientific community and among the general public. Epidemiological and basic research studies have shown that different components present in food such as tomatoes, olive oil, broccoli, garlic, onions, berries, soy bean, honey, tea, aloe vera, grapes, rosemary, basil, chili peppers, carrots, pomegranate and curcuma inhibit pro-proliferative signals implied in initiation and the progression of carcinogenesis, acting on pathways implied in cell proliferation, apoptosis and metastasis through their antioxidant, cytotoxic and pro-apoptotic properties (Kyle et al., 2010; Garavello et al., 2009; Aggarwal and Shishodia, 2006).

Vitamin C (L-ascorbic acid), is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component (Li and Schellhorn, 2007). Ascorbic acid is required for the biosynthesis of collagen, an essential component of connective tissue, which plays a vital role in wound healing (Carr and Frei, 1999).

Insufficient vitamin C intake causes scurvy, which is characterized by widespread connective tissue weakness, poor wound healing, hyperkeratosis, bleeding gums and loosening or loss of teeth due to tissue and capillary fragility (Stephen and Utecht 2001; Weinstein et al 2000). Deficiency symptoms appear only if vitamin C intake falls below approximately 10 mg/day for many weeks (Wang and Still 2007). Vitamin C deficiency is uncommon in developed countries but can still occur in people with limited food variety.

Recommended Intakes of ascorbic acid

Results from pharmacokinetic studies indicate that oral doses of 1.25 g/day ascorbic acid produce mean peak plasma vitamin C concentrations of 135 micromol/L, which are about two times higher than those produced by consuming 200ï 300 mg/day ascorbic acid from vitamin C-rich foods. Pharmacokinetic modeling predicts that even doses as high as 3 g ascorbic acid taken every 4 hours would produce peak plasma concentrations of only 220 micromol/L (Padayatty et al., 2004).

Intake recommendations for vitamin C is provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (Institute of Medicine, Food and Nutrition Board, 2000). Table 3 lists the current RDAs for vitamin C. For infants from birth to 12 months, the FNB established an adequate intake (AI) for vitamin C that is equivalent to the mean intake of vitamin C in healthy, breastfed infants.

Sources of Vitamin C

Food

Vitamin C is widely available in foods of both plant and animal origin, but the best sources are fresh fruits and vegetables. Vitamin C is found naturally in broccoli, cabbage, potatoes, peas, red peppers, brussel sprouts, kale, cauliflower, cantaloupe, strawberries, mangoes, tangerines, orange, grapefruit, lemons, and limes (Table 4). Although it is not naturally present in grains, it is added to some fortified breakfast cereals. However, because vitamin C is unstable when exposed to an alkaline environment or to oxygen, light and heat, losses may be substantial during storage and cooking (Weinstein et al., 2000). Steaming or microwaving may lessen cooking losses. Fortunately, many of the best food sources of vitamin C, such as fruits and vegetables, are usually consumed raw. Consuming five varied servings of fruits and vegetables a day can provide more than 200 mg of vitamin C.

Dietary supplements

Supplements typically contain ascorbic acid, which has equivalent bioavailability to that of naturally occurring ascorbic acid in foods, such as orange juice and broccoli (Bates 1997; Gregory, 1993). Other forms of vitamin C supplements include sodium ascorbate, calcium ascorbate, other mineral ascorbates, ascorbic acid with bioflavonoids and combination products, such as Ester-C®, which contains calcium ascorbate, dehydroascorbate, calcium threonate, xylonate and lyxonate (Bates 1997; Gregory, 1993).

Ascorbate fortification in food

Some cereals and beverages are fortified with ascorbic acid an odourless, white, crystalline compound which is stable in its dry form. Vitamin C is often added as a fortificant to fruit juices, fruit-flavoured drinks, juice added soda waters, dry cocktails or beverages, cereal-based products, and milk. Some juices like apple and grape that are not normally a source of vitamin C have vitamin C added to them. Most ready-to-eat cereals are also fortified with vitamin C. There are technologies available for vitamin C fortification of fruit juices, fruit juice drinks, other related beverages, dairy products and some breakfast cereals. For mixing with dry products, particle size and density are of course important considerations. A fat coated form of ascorbic acid is also available for enrichment purposes. High moisture content (greater than 7%) in the presence of oxygen is known to adversely affect the stability of vitamin C in cereals. Due to its high water solubility, losses due to leaching can be a problem in some processing procedures. In dehydrated citrus juices the degradation is dependent on both temperature and water activity. There is a need to develop more stable vitamin C compounds and evaluate their use in the fortification of a range of vehicles.

Since milk is low in iron and vitamin C content, it is desirable to fortify milk with them. Milk composition has been found to be improved with the addition of vitamin C, iron and zinc (Löker et al., 2003). Sharma, 2005 studied the stability of milk with microencapsulated vitamin C and found that the addition of vitamin C @ 30 mg/100 g was appropriate for fortification, because the acidity of milk did not increase noticeably and no negative effect on sensory quality was observed. In a study evaluating the stability of several forms of vitamin C during production and storage of white pan bread, the bread fortified with large crystals of ascorbic acid exhibited a higher retention of ascorbic acid by 20% than the bread fortified with small crystals of ascorbic

acid or with uncoated ascorbic acid (Park et al., 1994). General loss in vitamin C content during production of ready-to-eat cereals is approximately 37% (Steele, 1976). Studies have shown that encapsulation is the best way of protecting vitamin C. For the application in solid food systems (cereals, bread, and biscuits), spray-cooling, spray-chilling and fluidized bed appear to be the best form of encapsulation while in liquid food systems, liposomes represent the best way (De Zarn, 1995).

Role of vitamin C in cancer prevention

Metastatic cells proliferate rapidly; therefore they constantly require more energy to cope up with increased metabolism. During this course, reactive oxygen species (ROS) are excessively generated and lead to lipid peroxidation, DNA damage and consequently, apoptosis in cells (Scatena 2012; Azad et al 2010). Hence oxidative stress is a major apoptotic stimulus in cancer cells. However, by contrast, inhibition of oxidative stress also shows anticancer effects. Antioxidants exhibit a wide variety of biological functions, including apoptosis induction, growth arrest and inhibition of DNA synthesis (Di Domenico 2012; Hu 2011). Therefore, targeting the oxidative stress pathways through induction or inhibition, the generation of ROS may enhance the pro-apoptotic machinery of cancer cells and offer a novel strategy for treatment (Fig.2).

Epidemiologic evidence suggests that higher consumption of fruits and vegetables is associated with lower risk of most types of cancer, perhaps, in part, due to their high vitamin C content (Li and Schellhorn, 2007; Carr and Frei, 1999). Vitamin C can limit the formation of carcinogens, such as nitrosamines (Hecht 1997), in vivo; modulate immune response (Carr and

Frei, 1999); and, through its antioxidant function, possibly attenuate oxidative damage that can lead to cancer. Most case-control studies have found an inverse association between dietary vitamin C intake and cancers of the lung, breast, colon or rectum, stomach, oral cavity, larynx or pharynx, and esophagus (Jacob and Sotoudeh, 2002).

It has been found that high-dose vitamin C has beneficial effects on quality of life and survival time in patients with terminal cancer (Cameron and Pauling, 1976). Holly et al., (2014) suggested that dietary vitamin C intake was also statistically significantly associated with a reduced risk of total mortality and breast cancer specific mortality. In recent years the use of natural dietary antioxidants to minimize the cytotoxicity and the damage induced in normal tissues by antitumor agents is gaining consideration. Vitamin C has been shown to enhance the MTZ effect allowing the utilization of lower chemotherapic concentrations in comparison to the single treatments (Guerriero et al., 2014).

However, evidence from prospective cohort studies is inconsistent, possibly due to varying intakes of vitamin C among studies. Evidence from some randomized clinical trials suggests that vitamin C supplementation, usually in combination with other micronutrients, does not affect cancer risk. At this time, the evidence is inconsistent on whether dietary vitamin C intake affects cancer risk. A substantial limitation in interpreting many of these studies is that investigators did not measure vitamin C concentrations before or after supplementation. At daily intakes of 100 mg or higher, cells appear to be saturated and at intakes of at least 200 mg, plasma concentrations increase only marginally (Taylor et al., 1994). If subjectsô vitamin C levels were

already close to saturation at study entry, supplementation would be expected to have made little or no difference on measured outcomes (Padayatty and Levine, 2006).

Emerging research suggests that the route of vitamin C administration (intravenous vs. oral) could explain the conflicting findings (Bruno et al., 2006). Oral administration of vitamin C, even of very large doses, can raise plasma vitamin C concentrations to a maximum of only 220 micromol/L, whereas IV administration can produce plasma concentrations as high as 26,000 micromol/L (Hoffer et al., 2008). Concentrations of this magnitude are selectively cytotoxic to tumor cells in vitro (Li and Schellhorn, 2007). Research in mice suggests that pharmacologic doses of IV vitamin C might show promise in treating otherwise difficult-to-treat tumors and a high concentration of vitamin C may act as a pro-oxidant and generate hydrogen peroxide that has selective toxicity toward cancer cells (Chen et al., 2008).

Anticancer mechanisms of ascorbic acid

Anti-oxidant activity

Ascorbate is an important physiological antioxidant, very strong radical scavenger and has been shown to regenerate other antioxidants within the body, including alphatocopherol (vitamin E) (Jacob and Sotoudeh, 2002). Vitamin C reduces unstable oxygen, nitrogen, and sulfur radicals. In studies with human plasma, vitamin C protected plasma lipids against peroxidative damage induced by aqueous peroxyl radicals (Vandenberg et al 1990).

Ongoing research is examining whether vitamin C, by limiting the damaging effects of free radicals through its antioxidant activity, might help prevent or delay the development of

certain cancers and other diseases in which oxidative stress plays a crucial role. Vitamin C may act synergetically with tocopherol (vitamin E), the principal lipid soluble antioxidant (Niki, 1991). It reduces nitrates and prevents the formation of carcinogenic nitrosamines (Burton et al., 1990).

Pro-oxidant Property

Vitamin C possesses antioxidant activity, but it has cytotoxic activity at higher concentrations (González et al., 1998; Yamamoto et al., 1987). High-dose ascorbate bears cytotoxic effects on cancer cells in vitro and in vivo, making ascorbate a pro-oxidative drug that catalyzes hydrogen peroxide production in tissues instead of acting as a radical scavenger (Du et al., 2010; Chen et al., 2005).

Vitamin C can generate hydrogen peroxide upon oxidation which is enhanced by divalent cations such as iron and copper (Jonas et al., 1989). Hydrogen peroxide and its derivatives can compromise cell viability mainly by damaging the cell membranes and mitochondria. These oxidative reactions are limited in healthy organisms because most metal ions are bound to proteins in serum, which makes them unavailable to enhance the pro-oxidant activity of Vitamin C, while in malignancy, these ions are readily available (Gutteridge et al., 1980). These reactive species are capable of inducing multiple negative cellular effects such as DNA strand breaks, disruption of membrane function via lipid peroxidation, and depletion of cellular ATP (González et al., 1992).

Vitamin C mediated hydrogen peroxide generation is inhibited by serum due to certain proteins such as albumin and glutathione with antioxidant capacity or catalase, which decomposes hydrogen peroxide. Other antioxidant enzymes including glutathione peroxidase and superoxide dismutase complement the catalase enzymatic function (Sunil Kumar et al., 2010). Cellular damage through the accumulation of hydrogen peroxide and decreased levels of antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase have been presumed to be responsible for the selective toxicity of vitamin C in malignant cells (Riordan et al., 1995; Powers et al., 1994).

Vitamin C Oxidation Products

Furthermore, degradation products of ascorbic acid, such as dehydroascorbic acid, 2,3-diketogulonic acid, and 5-methyl 1-3,4-dehydroxytetrone, have demonstrated antitumor activity (Leung et al., 1993, Tsao et al., 1989; Tsao et al., 1988). These vitamin C oxidation products and/or metabolic by-products have a function in controlling mitotic activity. Dehydroascorbic acid mediates its anti-tumor activity by preventing cell division through inhibiting protein synthesis at the ribosomal level and its prolonged exposure may cause irreparable damage resulting ultimately in complete lysis of the cells (Grad et al., 2001).

Vitamin C and Intracellular Matrix

Ascorbic acid levels in cancer patients are significantly reduced suggesting an increase requirement for its anti-tumour mechanisms. Its deficiency causes generalized tissue disintegration involving the dissolution of intercellular ground substance and disruption of

collagen bundles (Kennedy, 1976). The stromal resistance may be a physical line of defense against cancer by encapsulating neoplastic cells with a dense fibrous tissue. This feature can be enhanced by high doses of vitamin C. The amount of collagen present determines the strength of the tissue and also its resistance to malignant infiltration.

Inhibition of Matrix Metelloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which can degrade the major components of Extra Cellular Matrix (ECM). Many reports have shown that ECM degrading enzymes, including MMPs, play a pivotal role in tumor invasion and metastasis (Sunil Kumar et al., 2013). A recent review suggested that MMP inhibitors have strong effects at the tumor promotion stage that may be, in part, associated with the inhibition of the expression of enzymes related to inflammation, such as COX-2 and iNOS, as well as angiogenic factors. Vitamin C down-regulates MMP-2 expression/activity in human amnion cultured cells, and the regulatory effect is exerted at the transcriptional level (Pfeffer et al., 1998).

Apoptosis-inducing activity

Vitamin C is known for its effective antioxidant potential under normal conditions (Sunil Kumar et al., 2010; Padayatty et al., 2003). However, it has also been found that at higher doses, vitamin C acts as a pro-oxidant, generating H_2O_2-dependent cytotoxicity in a variety of cancer cells in vitro and in vivo without adversely affecting normal cells (Chen et al., 2008; Chen et al., 2005). Some studies have shown that ascorbic acid in combination with other vitamins exert

necrosis like cell death independent of caspase-3, with a minor percentage of cell displaying mitochondrial depolarisation and DNA fragmentation (Verrax et al., 2005). However, the necrosis inducing action is due to ascorbate alone or the combination with other micro nutrients is not fully understood. Yet, the mechanism by which vitamin C-induces apoptosis in vitro is also not yet fully established. Few studies have reported that Vitamin C induces apoptosis in vitro through endoplasmic reticulum stress and mitochondrial pathway. In-vitro vitamin C treatment is found to increase expression of pro-apoptotic protein, Bax and tumor suppressor gene product, p53, which usually brings about apoptosis (Kim et al., 2007).

Anti-angiogenic effect

Angiogenesis involves formation of new blood vessels in both normal and cancerous tissues. Pathological angiogenesis is characterized by persistent proliferation of endothelial cells and blood vessel formation. This complex process plays an important role in tumor growth, invasion, and metastasis. In addition, circulating endothelial progenitor cells are involved in the development of vasculature, and many tumors are associated with bone marrow-derived endothelial cell infiltration (Conejo-Garcia et al., 2004; Direkze et al., 2004). High concentration of ascorbic acid has been found to suppress of angiogenesis in cancer models (Peyman et al., 2007). Varied effects of ascorbic acid supplementation on angiogenesis have been reported (Mikirova et al., 2008). High concentrations of ascorbic acid alter the metabolic activity of endothelial progenitor cells by decreasing the ATP levels. This further prevents significant endothelial cell proliferation thereby suppressing angiogenesis (Mikirova et al., 2008).

At higher concentrations vitamin C has been found to limit angiogenesis by inhibiting production of nitric oxide (NO), an important promoter of tumor angiogenesis. As endothelial NO formation depends on the presence of intracellular cofactors such as: NADPH, FAD, FMN and tetrahydrobiopterin (BH4), it is suggested that excessive ascorbate can change the oxidation-reduction status inside the cells. This could decrease the availability of nitric oxide, through the formation of peroxynitrite (Mikirova et al., 2008).

Other mechanisms

Vitamin C in high concentrations inhibits prostaglandins of the 2-series (arachidonic acid derived), which have been correlated with inflammation and increased cell proliferation (Beetens and Hermen, 1983). Inhibition of cell growth is also associated with the activation of transcription factor NF-kappa B by Vitamin C (Muñoz et al., 1997). Ascorbic acid along with vitamins K has been suggested to cause profound perturbations of cytoskeleton and membranes that ultimately kill the cells by a form of cell death (autoschizis) that is distinct from apoptosis, oncosis, or necrosis (Gilloteaux et al., 2001; Gilloteaux et al., 1998).

Large amounts of vitamin C intake may deplete the levels of certain amino acids in body fluids making these unavailable for rapidly growing tumors (Casciari et al., 2001; Riordan et al., 2000). Ascorbate is essential for immunoglobulin synthesis, active phagocytosis and also been shown to enhance interferon production (Siegel et al., 1975; Goetzl et al., 1974).

Conclusion

The data described above supports the exploration of vitamin D and ascorbic acid supplementation as approaches for cancer prevention and treatment. Various epidemiological data indicate that deficiency of these micronutrients is associated with an increased risk and poor prognosis of several types of cancer. Both these vitamins have prominent anti-proliferative, anti-angiogenic, pro-apoptotic and pro-differentiative effects in a broad range of cancers. Several clinical trials indicate that the administration of high-dose ascorbate and vitamin D analogues is safe and feasible. However, more extensive randomized controlled trials of these micronutrientsô supplementation, alone and in combination with cytotoxic and other anticancer agents, need to be conducted before making necessary recommendations as dietary supplements.

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Table 1: Selected Food Sources of Vitamin D (U.S. Department of Agricultutre, 2011)

Sl. No.	Food	International Units per serving	Percent Daily values*
1.	Cod liver oil, 1 tablespoon	1,360	340
2.	Swordfish, cooked, 3 ounces	566	142
3.	Salmon (sockeye), cooked, 3 ounces	447	112
4.	Tuna fish, canned in water, drained, 3 ounces	154	39
5.	Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	137	34
6.	Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124	29-31
7.	Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces	80	20
8.	Margarine, fortified, 1 tablespoon	60	15
9.	Sardines, canned in oil, drained, 2 sardines	46	12
10.	Liver, beef, cooked, 3 ounces	42	11
11.	Egg, 1 large (vitamin D is found in yolk)	41	10
12.	Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40	10
13.	Cheese, Swiss, 1 ounce	6	2

*DVs were developed by the U.S. Food and Drug Administration to help consumers compare the nutrient contents among products within the context of a total daily diet. The DV for vitamin D is currently set at 400 IU for adults and children age 4 and older.

Table 2: Recommended Dietary Allowances (RDAs) for Vitamin D (Institute of Medicine, Food and Nutrition Board, 2010)

Age	Male	Female	Pregnancy	Lactation
Below 1 year	400 IU (10 ε g)	400 IU (10 ε g)		
1-13 years	600 IU (15 ε g)	600 IU (15 ε g)		
14-18 years	600 IU (15 ε g)	600 IU (15 ε g)	600 IU (15 ε g)	600 IU (15 ε g)
19-50 years	600 IU (15 ε g)	600 IU (15 ε g)	600 IU (15 ε g)	600 IU (15 ε g)
51-70 years	600 IU (15 ε g)	600 IU (15 ε g)		
Above 70 years	800 IU (20 ε g)	800 IU (20 ε g)		

* The biological activity of 40 International units (IU) of vitamin D is equal to 1 ε g (microgram).

Table 3: Recommended Dietary Allowances (RDAs) for Ascorbic acid (Institute of Medicine, Food and Nutrition Board, 2000)

Age	Male	Female	Pregnancy	Lactation
0ï 6 months	40 mg*	40 mg*		
7ï 12 months	50 mg*	50 mg*		
1ï 3 years	15 mg	15 mg		
4ï 8 years	25 mg	25 mg		
9ï 13 years	45 mg	45 mg		
14ï 18 years	75 mg	65 mg	80 mg	115 mg
19 years and above	90 mg	75 mg	85 mg	120 mg

* Adequate Intake (AI)

Table 4: Selected Food Sources of vitamin C (U.S. Department of Agricultutre, 2011)

Sl. No.	Food	Milligrams (mg) per serving	Percent Daily values*
1.	Red pepper, sweet, raw, ½ cup	95	158
2.	Orange juice, ¾ cup	93	155
3.	Orange, 1 medium	70	117
4.	Grapefruit juice, ¾ cup	70	117
5.	Kiwifruit, 1 medium	64	107
6.	Green pepper, sweet, raw, ½ cup	60	100
7.	Broccoli, cooked, ½ cup	51	85
8.	Strawberries, fresh, sliced, ½ cup	49	82
9.	Brussels sprouts, cooked, ½ cup	48	80
10.	Grapefruit, ½ medium	39	65
11.	Tomato juice, ¾ cup	33	55
12.	Cabbage, cooked, ½ cup	28	47
13.	Cauliflower, raw, ½ cup	26	43
14.	Potato, baked, 1 medium	17	28
15.	Tomato, raw, 1 medium	17	28

*DV = Daily Value. DVs were developed by the U.S. Food and Drug Administration (FDA) to help consumers compare the nutrient contents of products within the context of a total diet. The DV for vitamin C is 60 mg for adults and children aged 4 and older. The FDA requires all food labels to list the percent DV for vitamin C. Foods providing 20% or more of the DV are considered to be high sources of a nutrient.

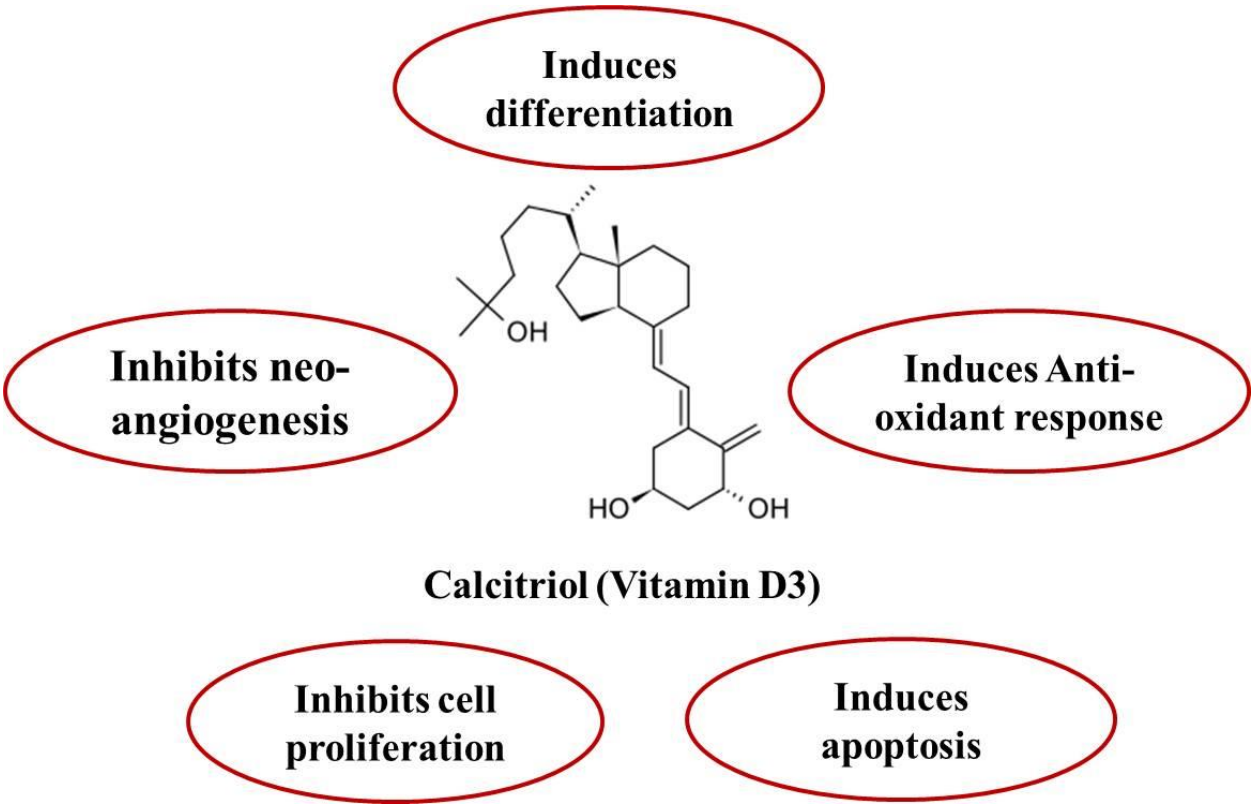

Fig.1 Anti-cancer effect of Vitamin D

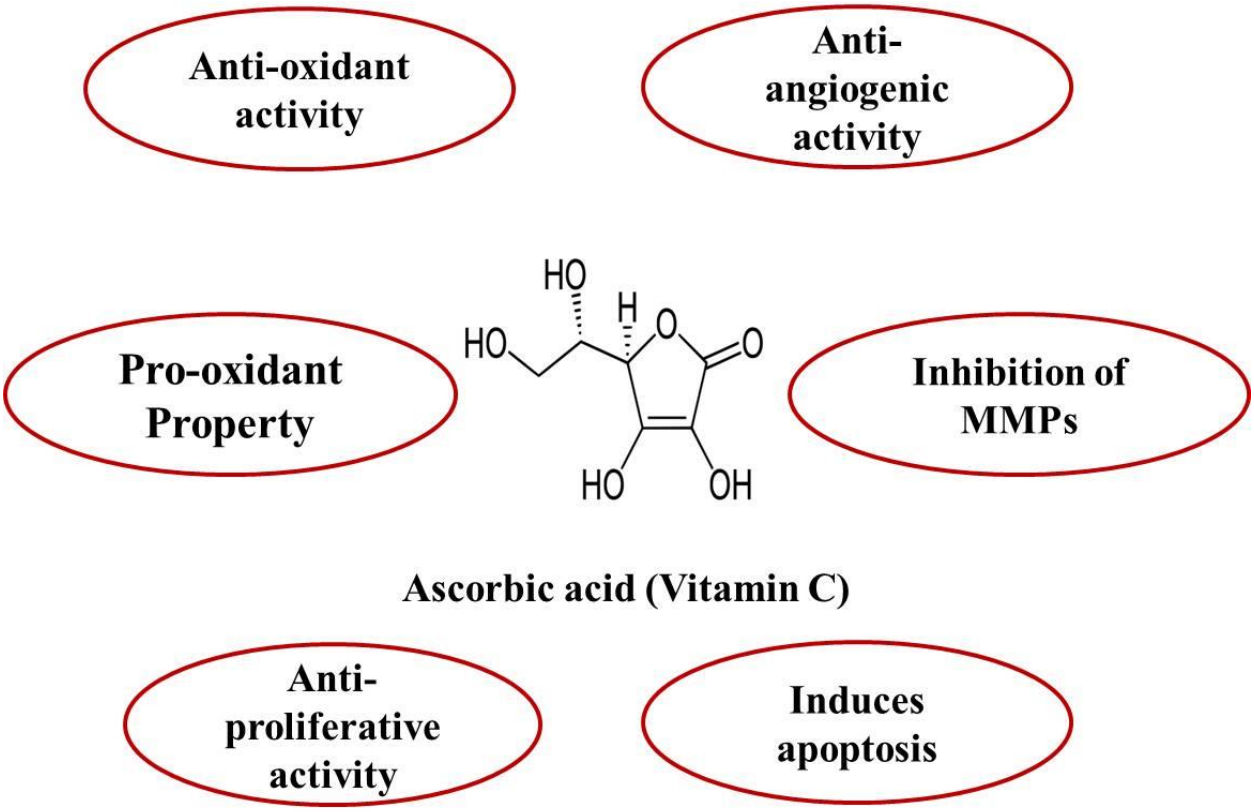

Fig.2 Anti-cancer effect of ascorbic acid