REVIEW ARTICLE

Anticarcinogenic Activity of Saponins with Special Reference to Diosgenin

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Introduction

Cancer, after cardiovascular disease, is the second leading cause of death (Kutluk and Kars, 1998; Odunsanya, 2001; Cetingoz *et al*, 2002; Turgay *et al*, 2005). Worldwide about 10 million people per year are diagnosed with cancer and more than 6 million die of the disease and over 22 million people in the world are cancer patients (Pinar, 1998; Steward and Kleihues, 2003).

Cancer is a complex disease that is normally associated with a wide range of escalating effects both at the molecular and cellular levels. It therefore seems unlikely that chemoprevention follows simplistic rules and formulations.

When cancer is diagnosed, therapists face a formidable range of challenges. Treatment usually consists of various combinations of surgery, radiation therapy, and chemotherapy but despite these therapeutic options, cancer remains associated with high mortality. Natural and some synthetic compounds can prevent, suppress, or reverse the progression of cancer. Although tumors have traditionally been treated with chemotherapeutic agents, the advents of compounds which prevent malignancies represent an emerging field and offer new options (Mann and DuBois, 2004).

Modern cancer therapies have significantly prolonged the life of the average cancer patient, but still have not succeeded in reducing cancer mortality in certain types of cancer (Amin and Mousa, 2007). Epidemiologic studies have suggested that diet is an important environmental factor involved in the etiology of the most prevalent forms of cancer, for example, colon cancer, breast cancer and pancreatic cancer.

Many studies show that high meat and fat consumption increase the risk of developing cancer, whereas consumption of diets high in cereals, fruits and vegetables reduces the risk (Amstrong and Doll, 1975, Jain *et al*, 1980, Kolonel *et al*, 1981, Manousos *et al*, 1983, Willett *et al*, 1990).

The first major meeting devoted to cancer prevention was organized by The American Association for Cancer Research in October 2002. With thousands of participants, this was a key event that brought cancer prevention to the forefront. The US Food and Drug Administration approved tamoxifen for reducing the risk of breast cancer and celecoxib for the prevention of familial adenomatous polyposis (Sabichi *et al*, 2003). The identification of these two drugs represents a paradigm shift in oncology from cancer treatment to cancer prevention.

The old saying "Prevention is always better than cure" is particularly true in the case of cancer where a cure, if at all possible, is associated with high cytotoxic loads and/or invasive procedures. With our growing understanding of the molecular etiology of cancer, it has become apparent that strategies which limit DNA damage and/or increase the probability of DNA repair by inhibiting aberrant proliferation will decrease cancer incidence (Bertram, 2001).

Primary cancer preventive strategies are those aimed at removing exposure to carcinogens, as a chemical in the case of tobacco, physical, in the case of UV exposure or mutifactorial in the case of diet and obesity. These preventive strategies have had mixed success. Some have been rather successful, such as the widespread elimination of asbestos, controls on aflatoxin contamination in foods and strict limitations on the amount of exposure to ionizing radiation. Success in primary prevention is reflected in the decreasing incidence of lung cancer, particularly in men, which parallel an earlier decrease in the consumption of tobacco (Bertram and Vine, 2005).

Secondary cancer prevention inhibits the consequences of carcinogen exposure. Unfortunately, due to the inappropriate intervention, dosage or, in the case of retinoids, unacceptable toxicity, this strategy has had only limited success in clinical situations.

Tertiary cancer prevention relies upon the identification and removal of pre-neoplastic lesions and the success

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of this approach can be readily observed with the dramatic decrease of uterine cancer resulting from the widespread screening for cervical cancer by the PAP test as from the early 1940's.

This approach is unfortunately limited to tissues that are easily accessed (Petignat *et al*, 2006). A variety of approaches have been employed in cancer chemoprevention. These include changes in diet, supplementation with specific vitamins and minerals, or administration of pharmacologic compounds. Investigators have identified approximately 400 drugs, vitamins, hormones and other agents that might help in preventing cancer. Clinical trials are underway to investigate an increasing number of agents (Amin and Mousa, 2007).

Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers like colorectal cancer. Phytochemicals derived from such fruits and vegetables, referred to as chemopreventive agents; include tannins, coumarins, lignans, quinones, stilbenes, curcuminoids, flavonoids and other groups of substances.

Various edible and medicinal plants have been extensively studied for their ability to inhibit tumor development. The use of traditional medicines is therefore, quite common in developing countries and is spreading rapidly in developed countries (Amin and Mousa, 2007).

The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while.

However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species were used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture (Joy *et al*, 2001).

Based on these studies worldwide, many health organizations in North America and around the world have formulated dietary guidelines as "Dietary Guidelines for Americans, 2010" (Released on January 31, 2011).

Plant foods contain, in addition to the traditional macronutrients, a wide variety of microcomponents such as enzyme inhibitors, phytosterols, indoles, flavones and saponins. These microcomponents are known to be biologically active. Their role in the prevention of chronic diseases is currently being investigated. Although the toxicological properties of plant saponins have long been recognized, there is renewed interest in these biologically active plant components because recent evidence suggests that saponins possess hypocholesterolemic (Oakenfull and Sidhu, 1990) and anticarcinogenic (Konoshima and Lee, 1986, Abe *et al*, 1987) activities.

Plant Phytochemicals

Several plants show anti-mutagenic as well as anticarcinogenic activities because of presence of phytochemicals (Al-Harbi *et al*, 1995). "Phyto" comes from the Greek word for "plant". Phytochemical are chemicals produced by plants, they are natural molecules produced to protect plant cells.

These phytochemicals not only protect the plant itself from solar radiation, disease organism or insect invasion, but consumption of these chemicals by humans has shown to keep cells in the body healthy and stable in many ways. These chemicals give plants their characteristic colour, flavor, smell and texture. They are found in abundance in frequently consumed foods such as fruits, vegetables, grains, legumes, and seeds (Huang *et al*, 1993).

Phytochemicals have complementary and overlapping mechanism of action, including of gene expression in cell proliferation, cell differentiation, oncogenes and tumor suppressor genes; induction of cell cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation and reduction; stimulation of the immune system; regulation of hormone metabolism; as well as antimicrobial and antiviral effects (Sun *et al*, 2002).

When oxygen is metabolized in the body, it can be converted to a highly charged form, a free radical. Free radicals will react with neighboring molecules, causing damage. Antioxidants act as free radical scavengers, defusing free radical damage, thus reducing wear and tear on our body. Although phytochemicals are not nutrients in the traditional sense, such as vitamins and minerals, researchers are actively studying their health benefits to consumers.

Table 1. List of phytochemicals

Name of Phytochemicals	Its Different Types	Imortant Plant source
1.Alkaloids	Caffeine Theobromine Theophylline	*Cola nitida* *Adhatoda vasica* *Aegle marmelos* *Cola pachycarpa* *Theobroma bicolor* *Tylophora asthmatica* *Alstonia scholaris*
2.Anthocyanins	Cyanidin Malvidin	*Citrus sinensis* *Vitis sp.* *Solanum melongena* *Primula sp.* *Rubus armeniacus*
3.Carotenoids	Beta-Carotene Lutein Lycopene	*Daucus carota* *Mangifera indica* *Carica papaya* *Raphanus sativus* In some fungi
4.Flavonoids	Epicatechin Catechins(Epigallocatechin gallate) Hesperidin Isorhamnetin Kaempferol Myricetin Naringin Nobiletin Proanthocyanidins Quercetin Rutin Tangeretin Resveratrol	*Camelia sinensis* *Foeniculum vulgare* *Pedalium murex* *Martynia annua* *Solanum lycopersicum* *Rubus fructicosus* *Spermacoce hispida* *Hemidesmus indicus* *Leptadenia reticulate* *Passiflora incarnata Linn.* *Amygdalus comminis* *Ginko biloba*
5.Hydroxycinnamic Acids	Chicoric acid Coumarin Perulic acid Scopoletin	*Aegopodium podagraria* *Petroselinum crispum* Terminalia chebula Punica granatum Rhus succedanea
6.Isoflavones	Daidzein Genistein	*Trifolium pratense* *Glycine max*
7.Lignans	Silymarin	Linum usitatissimum (flax), Podophyllum peltatum (Mayapple)
8.Monophenols	Hydroxytyrosol	*Lentinus edodes*
9.Monoterpenes	Geraniol Limonene	*Rosamarium officinalis* *Musa accuminata* *Rosamarium eriocalyx*
10.Organosulfides	Allicin Glutathione Indole-3-Carbinol Isothiocyanates Sulforaphane	*Brassica oleracea* *Allium sativum* *Wasabia japonica* *Mansoa alliance (garlic creeper)*
11.Phenolic Acids	Capsaicin Ellagic Acid Gallic acid Rosmarinic acid Tannic Acid Curcumin	*Camelia sinensis* *Rosemarinus officinalis* *Curcuma longa*
12.Phytosterols	Beta-Sitosterol	*Panax ginseng*
13.Saponins	dammaranes tirucallanes lupanes hopanes oleananes taraxasteranes ursanes cycloartanes lanostanes cucurbitanes diosgenin	*Saponaria officinalis* *Trigonella foenum graecum* *Polygala senega* *Bacopa monnieri* *Tribulus terrestris* *Panax ginseng* *Agave cantala*
14.Triterpenoids	Ursolic acid	*Crataegus pinatifida*
15.Xanthophylls	Astaxanthin Beta-Cryptoxanthin Beta-Cryptoxanthin	*Daucus carota*

Among the above mentioned phytochemicals saponins have received considerable attention in recent years, because of their various biological activities. In particular, their favorable anti-tumorigenic properties have attracted intensive research in developing saponins for tumor therapies.

Saponin as bioactive compound

Saponins are glycosides of steroids and triterpenoids that are widely distributed in terrestrial plants and in some marine organisms. The name saponin is derived from the Latin word 'sapo', which means the plant that consists of frothing agent when diluted in aqueous solution. Saponins comprise polycyclic aglycones.

The sapogenin or the aglycone part is either a triterpene or steroid. The combination of sapogenin, hydrophobic or fat-soluble, hydrophilic or water-soluble sugar part enhances the foaming ability of saponins. Some toxic saponins are known as sapotoxin (Lininger *et al*, 1998).

Chemistry of Saponins

Saponins are a structurally diverse class of compounds occurring in many plant species. They are characterized by a skeleton derived of the 30-carbon precursor oxidosqualene to which glycosyl residues are attached. Saponins are high-molecular-weight glycosides linked to a non-sugar aglycone. According to Hostettmann and Marston (1995), there are two ways to classify saponins.

A. Classification based on the aglycone

On the basis of aglycones saponins can be broadly divided into three types:

1. Triterpene glycosides
2. Steroidal glycosides
3. Steroidal alkaloid glycosides

Saponins on hydrolysis yield an aglycone known as "sapogenin". Saponin glycosides are divided into two types based on the chemical structure of their aglycones (sapogenins).

The so-called neutral saponins are derivatives of steroids with spiroketal side chains. The acid saponins possess triterpenoid structures.

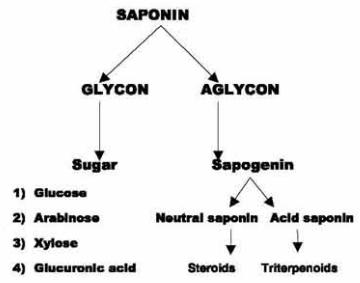


Fig.1. Composition of Saponin

The triterpene aglycone consists of a 30 carbon skeleton, synthesized from 6 isoprene units. The main pathway leading to both neutral and acidic types of sapogenins is similar and involves the head-to-tail coupling of acetate units. However, a branch occurs, after the formation of the triterpenoid hydrocarbon, squalene, that leads to steroids in one direction and to cyclic triterpenoids in the other (Fig 1 and 2).

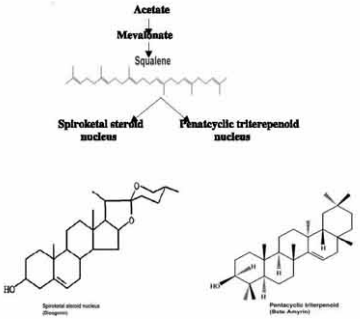


Fig.2. Pathways showing the synthesis of Seroids and Triterpenoids

The most commonly found ones are pentacyclic, followed by tetracyclic and hexacyclic structures. Pentacyclic skeletons include the oleananes (β-amyrin), ursanes (α-amyrin) and lupanes (lupeol). Over 50% of saponins isolated belong to β-amyrin type aglycone (Hostettmann and Marston, 1995).

The predominant tetracyclic aglycone is the dammarane type. Tetracyclic triterpene saponins act as intermediates in the synthesis of pentacyclic skeleton (Bruneton and Bruneton, 1995). Sugar chains are attached via 3-OH or 28-COO ester group. The aglycone can be further modified by multiple hydroxylations, unsaturation, etc.

B. Classification of saponins by the number of sugar chains

- Monodesmosidic saponins have one sugar chain attached, normally at C-3.
- Many saponins have other sugar chain attached either via an ester linkage at C-28 or an ether linkage at C-26 and they are termed bidesmosidic saponins.

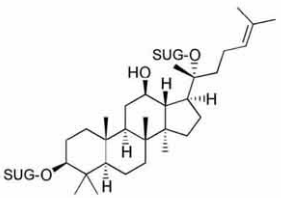


Fig.3. Structure of Protopanaxdiols a dammarane-type tetracyclic terpene sapogenin.

In this way, 11 main classes of saponins were distinguished: dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids. The dammaranes, lupanes, hopanes, oleananes, ursanes, and steroids are further divided into 16 subclasses, because their carbon skeletons are subjected to fragmentation, homologation, and degradation reactions. With this systematic classification, the relationship between the type of skeleton and the plant origin were investigated.

Diosgenin saponins are the most abundant and common steroidal saponins found in plants which exhibits cytotoxicity in several cancer cells. Diogenin has also been shown to induce apoptosis in HL-60 (human leukemia) cells7 and HeLa (cervical cancer) cells6 through activation of caspase-9 and 3, together with down-regulation of anti-apoptotic Bcl-2 protein (Jayadev *et al*, 2004).

Occurrence and Distribution of Saponins in Plants

Saponins are widely distributed in the plant kingdom, particularly in the angiosperms. In contrast, distribution of saponins in gymnosperms is very limited (Hostettmann and Marston, 1995). Triterpene saponins are mainly found in dicotyledons (Bruneton and Bruneton, 1995). Steroidal saponins are distributed in the monocotyledon families. Solanaceae, despite being a dicotyledonous plant family, contain steroidal saponins in all species studied so far (Sparg *et al*, 2004).

Steroidal alkaloid glycosides have narrower distribution range and Solanaceae and Liliaceae plant family act as the major sources for the same (Ikan, 1999). Steroidal saponins and steroidal alkaloid glycosides occurs concurrently in several species of Solanum, such as *S. khasianum* (Mahato *et al*, 1980).

Triterpene saponins are the most abundant saponins. Aglycones with a β-amyrin skeleton are the most common and are found in Curcubitaceae, Araliaceae, Asteraceae and Leguminosae (Charlwood and Banthorpe, 1991). These plants include food crops such as soy, cowpea and green bean (Kinjo *et al*, 1998). Tetracyclic triterpene algycones have relatively limited distribution. The well-studied saponins of this group are the ginsenosides. Ginsenosides are present in plants of the Panax genus including *P. ginseng, P. japonicus, P. notoginseng* and *P. quiquefolius,* which are all important herbs in folk medicine (Yang and Tanaka, 1999). Most triterpene saponins are mono or bidesmosidic. Steriodal saponins are also found in the members of Agavaceae, Alliaceae, Dioscoreaceae and Liliaceae family.

Spirostanol aglycone is mainly found in seeds, bulbs, or roots of plants; furostanol aglycone is found in the assimilatory parts (Hostettmann and Marston, 1995). However, furostanol glycosides are readily hydrolyzed by plant enzymes to give spirostanol glycoside during extraction, leading to their concurrent presence. Diosgenin, a six steroidal sapogenin isolated from Yams (*Dioscorea spp.*), is a commercially important source of starting material for production of semi-synthetic steroid hormones (Bruneton and Bruneton, 1995). Furostanol glycosides occur less widely in nature. *Smilax sp* used widely in Chinese medicine, contains a furostanol saponin sarsaparilloside. Steroidal alkaloid glycosides or glycoalkaloid have a much restricted distribution compared to triterpene and steroidal saponins. They occur mainly in the members of Solanaceae and Liliaceae. Steroidal alkaloid glycosides are found to be present in over 350 Solanum species, representing the richest natural source (Ikan, 1999). Important Solanaceae crops such as tomato, potato and eggplant all contain steroidal alkaloid glycosides. Another source of glycoalkaloids is the *Veratrum* species, which are mainly presented in the form of aglycone and glucosides.

Extraction and Purification of Saponin

The powdered sample of plant seed should be defatted by petroleum ether for 3 hours at 40°C by using soxhlet. After filtering the petroleum ether, extract the sample with methanol for 3 hours with mild heating; concentrate the methanol extract and re-extract with methanol and acetone (1:5 v/v) in soxhlet. Dry the precipitate obtained under vacuum, which turns to a whitish amorphous powder after complete drying. Load on Merck silica gel-60 (230-400 mesh) column and elute with chloroform-methanol-water (70:30:10).

Air dry the first fraction collected at room temperature (28°C) and treat the residue obtained as pure saponin. The purity of the saponin isolated can be analyzed by thin layer chromatography using chloroform and methanol (7:3) as the solvent system (Khanna and Kannabiran, 2009).

Other Methods for Extraction

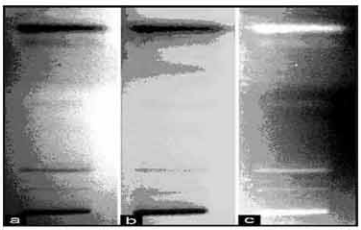
The general procedures of saponin extraction are as follows. First, the plant material is defatted by petroleum ether or hexane. Some authors include second defatting step with chloroform and ethyl acetate, but this has not been widely applied. Second extraction is often performed with methanol or aqueous methanol in dry and wet plant materials. MeOH: H_2O (3:2) (Endale *et al*, 2005), EtOH: H_2O (Verotta *et al*, 2002) are frequently used. The proportion of water included affects the saponins obtained, as methanol extracts contain bidesmosidic saponins but water extracts give monodesmosidic saponins (Domon & Hostettmann, 1984).Following evaporation, saponins are suspended in water and extracted with water saturated n-BuOH to obtain a saponin-rich extract (SRE).

An alternative method is to precipitate the saponins in large amounts of acetone or ether (Massiot *et al*, 1988). The precipitate is then dissolved in water and dialyzed against water in cellulose tubing under agitation for 72 hours, followed by freeze-drying. Elution with 40% MeOH removes interfering substances such as sugar and some phenolics. Saponins are then eluted out with pure methanol and further separated.

T.L.C Detection of Saponin

As the solvent system Benzene: methanol: acetic acid (90: 16: 8) rises, it carries the saponin in the spotted extracts on TLC plate to the various length based on their solubility.

Rf Values of Standard Saponin is 0.48 (Tullanithi, 2010).



a. Under visible light
b. Under UV long wavelength
c. Under UV-short wavelength

Fig.4. T.L.C detection of Saponins (Hullatti, 2011)

Table 2. HPLC systems for isolation of saponins

S.NO	Support	Mobile phase
1)	ODS gel	MeOH:H_2O MeCN: H_2O
2)	RP-8	MeOH: H_2O

Several studies illustrated the power of HPLC separation. Saponins differing in a single sugar unit can be resolved through HPLC (Reznicek *et al*, 1996).

The absence of chromophores moieties in saponin molecular structure and consequently its poor UV absorption is the major limitation to analyze this class of compounds using UV detection. Work was designed to develop a saponin extraction method, as well as a method, based on reverse-phase HPLC separation combined with UV detection, for saponin assay in Ilex paraguariensis extracts. The ursolic and oleanolic acids HPLC profile, detected at 203 nm, the peaks of the sapogenins in the saponin fraction appears at 15 min. Therefore, all saponins were hydrolyzed and the total concentration was expressed as ursolic acid (Gnotto *et al*, 2005).

Tahini Halvah which reflects plate flavour of Turkish is known specific of Turkish public in the world. Not only it is consumed in Turkey, but also it is consumed in lots of countries such as Germany, England, United States of America and Russian Federation. It is prepared from a mixture of sesame-seed paste (tahini) with inverted sugar syrup. The main components of Tahini Halvah are tahini sugar and the liquid extract of soapwort. Tahini is obtained by sesame seeds. Ceyhun et al., 2010 determined total saponin from Tahini Halvah by HPLC. Total saponin found to be 49-57% and 32-172 mg/kg in Tahini Halvah. The HPLC system (Shimadzu, Kyoto, Japan) that cosisted of a LC-10 AD-VP gradient pump, a Rheodyne 7125 i valve furnished with 20µL loop, a SPDM10A photodiode array detector, CTO-10AS column oven, DGU-14A degasser and a SCL-10A system controller. Seperation of saponins was carried out using a Nucleosil Macherey-Nagel C18 (250 x 4.6 mm ID, particle size 5µm) column (Barcelona, Spain) at 1.5 ml min G1 flow rate. Detection was made at 254 nm and 25°C. The HPLC mobile phase consisted of methanol, water and acetic acid in the ratio of 60/34/6 (v/v/v). The mobile was filtered and degassed prior to use. The compounds appering in chromotograms were identified on retention times and spectral data by comparasion with standards. Saponin that is active substance of soapwort extract affects positively the colour and consistency of the halvah and prevents especially the oozing of the oil from the halvah in time by acting an emulsifier.

Table 3. Chromatographic systems for isolation of saponins

S.No	Support	Mobile phase
1.	Silica gel	$CHCl_3$:MeOH:H_2O gradient EtOAc:EtOH gradient n-BuOH:EtOAc:H_2O gradient
2.	ODS gel	MeOH:H_2O gradient
3.	DiaionHP20	MeOH:H_2O gradient
4.	XAD-2	MeOH:H_2O gradient
5.	Sephadex LH-20	MeOH

Characteristics of Diosgenin: A Special Class of Saponin

Diosgenin is a steroidal sapogenin compound that is very useful in pharmaceutical industries as a natural source of steroidal hormones. Diosgenin is found in a few higher plant species and interest in its medicinal properties has increased recently (Liu *et al*, 2005).

Studies have found that diosgenin can be absorbed through the gut and plays an important role in the control of cholesterol metabolism (Roman *et al*, 1995).

Diosgenin produces changes in the lipoxygenase activity of human erythroleukemia cells and is responsible for morphological and biochemical changes in megakaryocyte cells (Nappez *et al*, 1995). Diosgenin is generally used as starting material for partial synthesis of oral contraceptives, sex hormones, and other steroids (Zenk, 1978).

The features of Diosgenin are as follows:

- Nature:-flaked or needle-like
- Colouration:-crystal Off white crystalline powder
- Melting range:-204-207 ° C.
- Boiling point:-527° C.
- Vapour pressure:- 3 x 10^{-13} mmHg (at 25° C)
- Specific heat of vapourization :- 0.222 Kj/g
- Optical rotation:- 120 degree to 122 degree
- Loss on drying:- 0.2% to 0.5%
- Assay:- 90% to 92.5%.
- Molecular Formula:- $C_{27}H_{42}O_3$
- Molecular Weight:- 414.627
- CAS No.:- 512-04-9
- Solubility:- in gasoline, ethanol, chloroform and other organic solvents and do not dissolve in water.

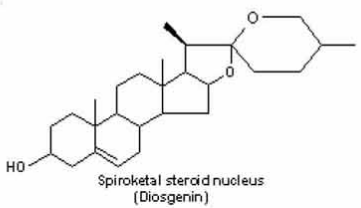


Fig.5. Structure of Diosgenin

Chemistry of Diosgenin

Hydrolysis of the dioscin (saponin) leads to scission of the trisaccharide at the 3-position and the formation of the aglycone, diosgenin. Treatment of this acetal with hot acetic anhydride in the presence of toluenesulfonic acid leads initially to protonation of one of the acetal oxygens followed by elimination to form enol ether. Oxidation by means of chromium trioxide leads to preferential attack at the electron-rich enol ether double bond. In effect, this transformation converts the side chain at C-17 in diosgenin to the acetyl group required for many steroid drugs. Heating that intermediate in with alcoholic sodium hydroxide leads to the elimination of the ester grouping beta to the ketone; there is thus obtained 16-dehydropregnenolone acetate. The presence of the olefin at C-17 allows ready entry to C-19 androstanes and provides the necessary function for the synthesis of potent C-16- and C-16, 17-substituted corticosteroids (Marker *et al*, 1947).

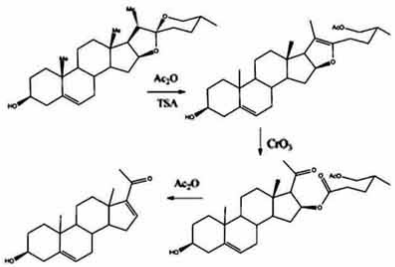


Fig.6. Hydrolysis of Saponins resulting in Diosgenin (Marker, 1947)

Mechanism of Action

Herbs and spices are known to exhibit an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that are believed to contribute to their anticarcinogenic and antimutagenic activities.

Experiments to test the chemopreventive efficacy of other spices are only at the initial stages. It is quite apparent that almost all the spices which are being commonly used are potent antioxidative agents, i.e., they can neutralize free radicals and protect our body against oxidative damage. Unless protected by antioxidants, macromolecules such as RNA, DNA, proteins, and lipids are damaged by free radicals. Increasing evidences revealed that oxidative damage is involved in the pathogenesis of carcinogenesis. Oxidative mechanisms plays a potential role in different stages of carcinogenesis such as initiation, promotion, and malignant conversion (progression).Thus, anti-oxidants, by virtue of their capability to quench free radicals can prevent oxidative damage to DNA, thereby decreasing the frequency of deleterious mutations.

Dietary constituents can also exert their beneficial effect by modifying drug metabolism and transport and thereby contributing to inter individual variability in drug disposition. In fact, it is now well established that nutrients and phytochemicals can have pronounced impact on drug disposition.

Mechanisms involved in the inhibition of cell proliferation and induction of apoptosis from Fenugreek diosgenin are recognized as being pivotal. Bcl-2 and caspase-3 among others have been implicated as molecular mediators of apoptosis (Goyal, 2001; Cory *et al*, 2003). Colon tumors are characterized by an overexpression of bcl-2, whereby apoptosis is down-regulated (Hirose *et al*, 1997), and chemopreventive agents decrease the expression of bcl-2 in human colon cancer cells, thus augmenting apoptosis (Ocker *et al*, 2003; Levy *et al*, 2003). By contrast, the proapoptotic caspase-3 is down-regulated in colon tumors, and its expression is increased by cancer preventive agents (Goyal *et al*, 2001). Several studies have used the measurement of apoptosis in colon cancer cells induced by chemopreventive agents to assess the efficacy of such agents (Ocker *et al*, 2003; Hong *et al*, 2007). Whether diosgenin inhibits colon tumor cell growth by the induction of apoptosis is not known. Dietary fenugreek seed and its major steroid saponin constituent diosgenin have a role in inhibiting or retarding ACF formation during initiation/postinitiation and promotional stages of azoxymethane-induced rat colon carcinogenesis. In addition, the effect of diosgenin on inhibiting cell growth and modulating the expression of bcl-2 and caspase-3 in HT-29 human colon cancer cells was also determined.

Ginsenosides such as Rh2 and Rg5 have been shown to induce G1 phase arrest in human MCF-7 breast cancer cells. Apoptosis can also be induced in human SKHEP-1 hepatoma cells by Rh2 through activation of cyclin A/cyclindependent kinase cdk 2 activity and caspase-3-mediated p21 cleavage. Astragalus saponins (AST) inhibit cell proliferation through accumulation in S phase and G2/M arrest, with concomitant suppression of p21 expression and inhibition of cyclindependent kinase activity. Besides, AST promotes apoptosis in HT-29 cells through caspase-3 activation and poly (ADP-ribose) polymerase cleavage, which is indicated by DNA fragmentation and nuclear chromatin condensation (Mandy *et al*, 2005).

Diosgenin induced apoptosis in HT-29 cells at least in part by inhibition of bcl-2 and by induction of caspase-3 protein expression (Bhattacharjee *et al*, 2009).

Pharmacological Properties

Hypoglycemic activity

Consumption of commercial diosgenin demonstrated hypoglycemic properties, which are beneficial in diabetes by reducing intestinal disaccharide activities. It has been reported using experimental studies in diabetic male Wistar rats, where there is a significant increase in lactase and maltase activities, reduced intestinal sucrose activity and also the activity of glucose -6- phosphate was significantly increased (Marie *et al*, 2006).

In obesity, adipocyte hypertrophy and chronic inflammation in adipose tissues cause insulin resistance and type-2 diabetes. Trigonella foenum-graecum (fenugreek) can ameliorate hyperglycemia and diabetes. Fenugreek miniaturized the adipocytes and increased the mRNA expression levels of differentiation-related genes in adipose tissues. Fenugreek ameliorated diabetes by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues, and its effects are mediated by diosgenin. Fenugreek containing diosgenin may be useful for ameliorating the glucose metabolic disorder associated with obesity (Uemura *et al*, 2010).

The in vitro study on glucose utilization by isolated rat hemi diaphragm suggest, that the aqueous extract of Solanum xanthocarpum may have direct insulin like activity due to presence of saponin which enhances the peripheral utilization of glucose and have extra pancreatic effects (Kar *et al*, 2004).

Diabetic rats (fed supplement and unsupplemented diets) lost weight significantly compared to the normal groups and there was a significant increase in the activity of alpha- amylase in the proximal region of the small intestinal mucosa of diabetic rats feeds sapogenin extract or commercial diosgenin (Mc Anuff *et al*, 2004).

Hypolipidemic and Antioxidant activity

Oxidative stress has been suggested as a main risk factor in the development of atherosclerosis. Diosgenin enhanced the resistance to lymphocyte DNA damage caused by an oxidant challenge with H_2O_2. The hypolipidemic and antioxidative effects on rats fed with a highcholesterol diet supplemented with either 0.1% or 0.5% diosgenin for 6 weeks has been investigated. Diosgenin showed a decrease in the plasma and hepatic total cholesterol levels (Tn suk *et al*, 2007).

High cholesterol diet in rats exhibited significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C. Antihyperlipidemic effects in lipid profile were exhibited in Fenugreek ethanol extract (FE), Catechu ethyl acetate extract (CE) and its low and high dose combination. Combined therapy of FE and CE showed higher anti hyperlipidemic effect than the individual FE and CE. Hypolipidemic activity of FE may be attributed due to presence of steroidal saponins, alkaloids and free amino acids (Patel *et al*, 2011).

Costus speciosus Koen. (Keu, Crape ginger), an Indian ornamental plant, has long been medicinally used in

traditional systems of medicine. It is valued very much for its diosgenin content. Costus is one of the plants, which contain diosgenin in its rhizome. The plant has been found to possess diverse number of pharmacological activities like anti-inflammatory, antiarthritic effect and antifungal activities (Srivastava *et al*, 2011). Vijayalakshmi and Sarada, 2008 investigated different parts of Costus speciosus for their polyphenol content and antioxidant activity.

Chakraborty, 2009 showed the antioxidant activity of chloroform extract of Costus speciosus leaves for its free radical scavenging activity.

Neuroprotective activity

In-vitro studies showed that HIV proteins, gp120 and Tat, Tat+morphine caused increased neurotoxicity in human neuronal cultures with ApoE4 allele. A number of novel antioxidants has been screened and found that only L-deprenyl and diosgenin protected against the neurotoxicity of Tat+morphine (Chun *et al*, 2007).

Expriments were conducted on neuroprotective effect of diosgenin on the senescent mice induced by D-galactose (D-gal). In this experiment mice were orally administered with diosgenin (1, 5, 25 and 125 mg/kg), for four weeks from the sixth week. The learning and memory abilities of the mice in Morris water maze test and the mechanism involved in the neuroprotective effect of diosgenin on the mice brain tissue were investigated. These results indicated that diosgenin has the potential to be a useful treatment for cognitive impairment. In addition, the memory enhancing effect of diosgenin may be partly mediated via enhancing endogenous antioxidant enzymatic activities (Chiu *et al*, 2011).

22R-Hydroxycholesterol is an intermediate in the steroid biosynthesis pathway shown to exhibit a neuroprotective property against β-amyloid (1-42) (Aβ) toxicity in rat PCl2 and human NT2N neuronal cells by binding and inactivating Aβ (Leeanu *et al*, 2003).

Vasodilating activity

Diosgenin is structurally "fairly similar" to progesterone. It is the precursor for the industrial large scale synthesis of different hormones including progesterone and nor ethisterone (Lisias, 2007).

Oxidative stress, as a main risk factor causes vascular endothelial cell apoptosis, which is implicated in the pathogenesis of cardiovascular disorders. Diosgenin, an aglycone of steroidal saponins, has been reported to exert anti-proliferative and proapoptotic actions on cancer cells widely. It has been proposed that diosgenin can protect the hyperlipidemic rats and prevent endothelial apoptosis under oxidative stress (Gong *et al*, 2010)

Role in Cholesterol Metabolism

Diosgenin, structurally similar to cholesterol, has been shown to decrease cholesterol absorption and to increase biliary cholesterol secretion without altering either serum cholesterol or total biliary bile salt secretion (Andrew *et al*, 1993).

Role in Melanogenesis

An increased level of melanin is characteristic of a large number of skin diseases, including acquired hyperpigmentation conditions such as melasma, post inflammatory melanoderma and solar lentigo. Diosgenin inhibits the melanin content significantly (Jongsung *et al*, 2007).

Diosgenin, at doses up to 50μM inhibited melanogenesis in B16/ BL6 murine melanoma cells; however even at the highest dose of 50μM studied, no cytotoxicity was observed in these cells (Lee *et al*, 2007).

Role of Diosgenin in Cell Cycle Arrest and Apoptosis in Cancer Cell Lines

Treatment of tumor cells with cytotoxic agents usually results in the breakdown of the cell cycle machinery, the cells subsequently entering into programmed cell death or apoptosis. Diosgenin plays a significant role in apoptosis. Diosgenin can inhibit proliferation via blocking cell cycle progression at the G2/M phase and subsequently progression to apoptosis in human leukemia K562 cells. Diosgenin can effectively inhibit the viability and proliferation of breast cancer cells MCF-7(Jia *et al*, 2007). Diosgenin induces differentiation of human erythroleukemia cell line (HEL TIB 180) through changing lipoxygenase activities (Yannick, 2005).

Diosgenin induced G2/M arrest of cell cycle progression through p21 up-regulation in a p53-independent pathway and strong induction of apoptosis in HEL cells. Apoptosis induction was accompanied by an increase in Bax/Bcl-2 ratio, PARP cleavage and DNA fragmentation (Leger *et al*, 2004)

It was observed that diosgenin, altered cell cycle distribution and induced apoptosis in the human osteosarcoma 1547 cell line (Corbiere *et al*, 2004).

Adverse Effect

As an herbal extract, diosgenin appears to be free of any major adverse effects (Benghuzzi *et al*, 2003).

Saponins as antimutagenic and anticarcinogenic agent:

In recent years, saponins have received considerable attention because of their various biological activities. Many experiments revealed the fact that saponins from different plants have antimutagenic and anticarcinogenic properties.

Among several herbs and spices fenugreek (*Trigonella foenum graecum*) and its active constituents possess potential anticarcinogenic activity. Studies evaluated the preventive efficacy of dietary fenugreek seed and its major steroidal saponin constituent, diosgenin, on azoxymethane-induced rat colon carcinogenesis during initiation and promotion stages. Preneoplastic colonic lesions or aberrant crypt foci (ACF) were chosen as end points. To evaluate the effect of the test agent during the initiation and postinitiation stages, 7-week-old male F344 rats were fed experimental diets containing 0% or 1% fenugreek seed powder (FSP) or 0.05% or 0.1% diosgenin for 1 week and were injected with azoxymethane (15 mg/kg body weight). Effects during the promotional stage were studied by feeding 1% FSP or 0.1% diosgenin 4 weeks after the azoxymethane injections. Rats were sacrificed 8 weeks after azoxymethane injection, and their colons were evaluated for ACF. In comparison with control, continuous feeding of 1% FSP and 0.05% and 0.1% diosgenin suppressed total colonic ACF up to 32%, 24%, and 42%, respectively. Dietary FSP at 1% and diosgenin at 0.1% fed only during the promotional stage also inhibited total ACF up to 33% and 39%, respectively. Importantly, continuous feeding of 1% FSP or 0.05% or 0.1% diosgenin reduced the number of multicrypt foci by 38%, 20%, and 36% by comparison with the control assay. In addition, 1% FSP or 0.1% diosgenin fed during the promotional stage caused a significant reduction of multicrypt foci compared with control. Dietary diosgenin at 0.1% and 0.05% inhibited total colonic ACF and multicrypt foci formation in a dose-dependent manner. Results from in vitro experiments indicated that diosgenin inhibits cell growth and induces apoptosis in the HT-29 human colon cancer cell line in a dose-dependent manner (Jayadev *et al*, 2011).

The effect of diosgenin from fenugreek on migration and invasion in human prostate cancer PC-3 cells was reported. Diosgenin inhibited proliferation of PC-3 cells in a dose-dependent manner. When treated with non-toxic doses of diosgenin, cell migration and invasion were markedly suppressed. Furthermore, diosgenin reduced the activities of matrix metalloproteinase-2 (MMP-2) and MMP-9 by gelatin zymography assay. The mRNA level of MMP-2, -9, -7 and extracellular inducer of matrix metalloproteinase (EMMPRIN) were also suppressed while tissue inhibitor of metalloproteinase-2 (TIMP-2) was increased by diosgenin. In addition, diosgenin abolished the expression of vascular endothelial growth factor (VEGF) in PC-3 cells and tube formation of endothelial cells. Immunoblotting assays indicated that diosgenin potently suppressed the phosphorylation of phosphatidylinositide-3 kinase (PI3K), Akt, extracellular signal regulating kinase (ERK) and c-Jun N-terminal kinase (JNK). In addition, diosgenin significantly decreased the nuclear level of nuclear factor kappa B (NF-κB), suggesting that diosgenin inhibited NF-κB activity. The results suggested that diosgenin inhibited migration and invasion of PC-3 cells by reducing MMPs expression. It also inhibited ERK, JNK and PI3K/Akt signaling pathways as well as NF-κB activity. These findings reveal new therapeutic potential for diosgenin in anti-metastatic therapy (Chen *et al*, 2011).

Cytotoxicity assay results indicated the anticancer therapeutic property of the root extract of Jatropha curcas Linn. containing saponins against human colon adenocarcinoma (HT-29) cell line but its cytotoxic effect on human hepatocyte (Chang cell) was high. The Root extract of Jatropha curcas appeared to be more active compared to leaf and stem bark on both cell lines. Interestingly, 25 µg/ml of root methanolic extract decreased the HT-29 cell viability to 28.8% while the Chang liver cell viability was 72.4%. The IC50 concentration for HT-29 and Chang liver cell lines were 18.3 ± 0.98 and 33.3 ± 0.75µg/ml respectively (Oskoueian *et al*, 2011).

Saponins of *Paris polyphylla* var. *yunnanensis* have anticarcinogenic activity. Eight known steroid saponins were isolated from the rhizome of *Paris polyphylla*. The LA795 lung adenocarcinoma cell line from mice was chosen to evaluate cytotoxicity by means of MTT assay, and to study apoptosis by means of AnnexinV-FITC/PI flow cytometry. Diosgenin-3alpha-L-arabinofuranosyl (1-->4)-[alpha-L-rhamnopyranosyl(1-->2)]-beta-D-glycopyranoside (compound 1), the main steroid saponin of *Paris polyphylla* and diosgenin (Dio), were evaluated for antitumor activity on LA795 lung adenocarcinoma in T739 inbred mice. Saponins showed remarkable cytotoxicity and caused typical apoptosis in a dose-dependent manner. They were evaluated in vivo by their effect on tumor developed in T739 inbred mice. The oral administration to T739 mice bearing LA795 lung adenocarcinoma of compound 1 and

diosgenin significantly inhibited tumor growth, by 29.44% and 33.94%, respectively. Hematoxylin Eosin (HE) staining showed that lungs and livers of treated mice underwent various levels of histo-pathological alterations. It was demonstrated by TUNEL assay that apoptosis rate in tumor cells was increased in comparison to cells in control mice (Yan *et al*, 2009).

The anticancer-cytotoxic activities of isolated saponins, gymnemagenol ($C_{30}H_{50}O_4$) from *Gymnema sylvestre* and dasyscyphin C ($C_{28}H_{40}O_8$) from *Eclipta prostrata* leaves were tested under in vitro conditions in HeLa cells. The gymnemagenol and dayscyphin C at 50µg/ml showed a good cytotoxic activity (63% and 52%, respectively) in HeLa cells at 48 hours with the IC_{50} value of 37 and 50µg/ml, respectively. 5-Fluorouracil (5-FU), a positive control, showed 57.5 % cell death with the IC_{50} value of 36µg/ml. The percentage of HeLa cell death was maximum (73%) after 96 hours with gymnemagenol, whereas dasyscyphin C showed only 53%. The isolated saponins were not toxic to Vero cells. From this study, it can be concluded that the saponins, gymnemagenol, and dayscyphin C have significant anticancer-cytotoxic activity on HeLa cells under in vitro conditions (Khanna and Kannabiran, 2009).

The antitumor activity of the saponin (diosgenin) isolated from the roots of *Pulstailla koreana*, on BDF-1 mice bearing Lewis lung carcinoma (LLC) cells as well as its cytotoxic activity against some cancer cell lines were evaluated. The Pulsatilla genus, *Pulsatilla koreana* Nakai (Ranuculaceae) in Korea is an important herb in traditional medicine that has been used to treat amoebic dysentery and malaria (Bae, 1999). The 50% aqueous EtOH fraction (WT fraction) of the roots of P. koreana was evaluated in a BDF1/LLC animal model. The WT fraction was injected at the maximum tolerated dose of 280 mg/kg/day and Adriamycin, which was used as the positive control, was administered at 0.5 mg/kg/day to the right groin of the BDF1 mice bearing the LLC cells. The results expressed that the WT fraction exhibited significant antitumor activity with an IR of 56 % (day 14) and 55 % (day 15), although it was less than that of adriamycin (60% and 64%) (Kim *et al*, 2004).

Ginseng is used as an agent for longevity, might be effective in preventing and suppressing cancer. In 1980 at Third International Ginseng Symposium (Seoul), T. K. Yun and his coworkers reported the anticarcinogenic activity of orally administered red ginseng extracts to ICR mice (Yun et al., 1999). As the carcinogens, 9, 10-dimethyl-1, 2-benzanthracene (DMBA), urethane, N-2 fluorenylacetamide (FAA), and aflatoxin B1 were respectively injected to the subscapular area of mice. After 26 weeks of DMBA injected group, no significant inhibitory effect of red ginseng extracts against adenoma was observed, but proliferation of lung adenoma was inhibited by 23% after 48 weeks. At the 28 weeks of urethane-injected group, red ginseng extracts decreased the incidence of lung adenoma by 22% and that of multiplicity by 31%. In the experiment by using FAA as a carcinogen, red ginseng extracts showed no significant inhibitory activity. At the 56 weeks of aflatoxin B1 injected group, red ginseng extracts decreased the incidence of lung adenoma by 29% and that of hepatoma by 75%. These pre-clinical experiments demonstrated the anticarcinogenic activity of red ginseng extracts. Subsequently, Yun in 1995 performed epidemiological studies among Korean people and demonstrated the non-organ-specific cancer-preventive activity of ginseng extracts. He investigated the cancer-preventive effect of ginseng in case-control studies, in which the number of subjects was extended from 905 to 1987 pairs. In both the epidemiological studies, odd ratios of white ginseng powder intake (0.44, 0.30) and red ginseng extracts intake (0.45, 0.20) were remarkably reduced, and cancers were non-organ-specific. The intake of fresh ginseng slices, fresh ginseng juice, and white ginseng tea did not decrease the risk for cancer. However, as the odds ratios show, the risk for cancer was rather unexpectedly low in the cases of intake of 1-3 times/year (0.62), 4-11 times/year (0.48), and 1 time/ month or more (0.31). Overall, the risk for cancer decreased as the frequency and duration of ginseng intake increased. The total lifetime intake of ginseng (301-500) gave 0.33 odds ratios for male and 0.29 for female, which shows the dose-response relationship (Shibata, 2001).

Another study shows that ginseng extract inhibits the growth of different types of tumors (Lee and Huemer 1971, Yun *et al*, 1983, Ha and Lee 1985, Odashima *et al*, 1985, Kenarova et al., 1990). The biological activity in ginseng is largely attributed to the triterpenoid saponins (ginsenosides), which constitute 2-4% of ginseng's dry weight. Growth inhibition and reverse transformation of B16 melanoma cells were observed with ginsenosides treatment (Odashima *et al*, 1985). Specific ginsenosides, Rh1 and Rh2, are extracted from the root of *Panax ginseng* (C. A. Mayer, personal communication). Although the only difference between the two saponins is the sugar moiety binding site, their biological effects were significantly different. Rh2 decreased the growth of cells while stimulating melanogenesis and cell-to-cell adhesiveness. Tubeimoside I, a triterpenoid saponin from the bulb of *Maxim franquet* was tested for activity against inflammation and tumorigenesis (Yu *et al*, 1992). A significant dose-dependent inhibition of edema induced by arachidonic acid and tissue plasminogen activator (TPA) was recorded. Tubeimoside I was also found to significantly decrease the number of tumor-

bearing mice. Saponin extracted from *Gleditsia japonica* effectively inhibited the growth of mouse skin papilloma that was induced and promoted by DMBA and TPA respectively without any toxic effect (Tokuda *et al*, 1991).

A Chinese herbal drug, Yunan Bai Yao, has been used as a hemostatic agent and is known to promote wound healing. digitonin saponin, formosanin-C, extracted from Liliaceae and also a component of Yunan Bai Yao, has been shown to have antitumor activity that acts by modifying the immune system (Wu *et al*, 1990). Extracts of Yunan Bai Yao exhibited cytotoxic activity in several cancer cell lines when a tissue culture screen was used. The cytotoxic component of this herbal medicine was later identified as the saponin formosanin-C (Ravikumar *et al*, 1979). Formosanin-C injected intraperitoneally inhibited the growth of hepatoma cells implanted in mice. Blood samples from these animals showed that the activity of natural killer cells and the production of interferon were significantly increased. The ginsenoside from the root of *Panax ginseng* was shown to increase both humoral and cell mediated immune responses (Kenarova *et al*, 1990). Spleen cells recovered from ginsenoside-treated mice injected with sheep red cells as the antigen showed significantly higher plaque-forming response and hemagglutinating antibody titer to sheep red cell antigen. It increased the number of antigen reactive T helper cells and T lymphocytes. There was also significant increase in natural killer activity and lymph node indexes in gisenoside-treated animals.

Saponins also delay the initiation and progression of cancer. Metabolic epidemiological studies have shown a strong association between colon cancer incidence and a high concentration of cholesterol metabolites and bile acids in the feces (Reddy and Wynder, 1973; Mower *et al*, 1979). Animal studies also support this relationship between secondary bile acids and colon carcinogenesis (Narisawa *et al*, 1974; Reddy *et al*, 1976, 1977). Repeated intrarectal dose of lithocholic or taurodeoxycholic acid increased the frequency of N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-induced colorectal neoplasms in rats (Narisawa et al., 1974). Also, weekly intrarectal doses of deoxycholic acid for 52 week increased the number of MNNG-induced colon adenocarcinomas (Reddy *et al*, 1976). Similarly, intrarectal injection of sodium cholate or sodium deoxycholate increased the incidence of adenomas and adenocarcinomas in rats (Reddy *et al*, 1977). These observations are important because there is convincing evidence of an interaction between saponins and bile acids (Malinow *et al*, 1979, Sidhu and Oakenfull, 1986).The free form of bile acids in the upper gastrointestinal tract and lower the absorption of bile acids across the mucosa as well as the formation of secondary bile products from primary bile acids. Increases in the fecal excretion of steroids, especially bile acids, were observed after feeding mice semi-synthetic diets containing 1% soybean saponins (Sidhu and Oakenfull, 1986). A similar increase in fecal biliary excretion was observed during ingestion of diets containing alfalfa seeds (Malinow *et al*, 1979). These results suggest that saponins from different dietary sources reduce the availability of bile acids to form secondary bile acids by intestinal microflora and therefore may prevent the development of colon cancer.

Soybean saponins are considered to be neutral saponins. Hypocholesterolemic effects of soybean saponins have been demonstrated by several investigators. Isolated soybean saponins reduced diet-induced hypercholesterolemia in rats through an increase in bile acid excretion (Oakenfull *et al*, 1981). They also form complexes with bile acids and reduce their absorption in vitro (Sidhu and Oakenfull, 1990). The anticarcinogenic properties of soybean saponins are thought to be related to their ability to bind with bile acids (cytotoxic compounds and tumor promoters). After saponin administration, neither saponin nor sapogenin was detected in the blood. However, sapogenins were recovered from the cecum and colon of the animals (Gestetner *et al*, 1968). It is likely, therefore, that, in the intestine, saponins bind to the mucosal cell membrane and change its physiology. Because the membranes of some cancer cells contain more cholesterol than do normal cell membranes (Hilf *et al*, 1970, Perkins and Kummerow, 1976), it is possible that saponins bind more to cancer cells and as a result induce their destruction.

Mice fed with cholic acid alone showed increased colonic epithelial cell proliferation and those mice fed diets containing cholic acid and 1% saponin showed normal cell proliferative characteristics. During the neoplastic process of colonic epithelial cells, major zones of DNA synthesis for cell proliferation are extended from the normal crypt base to the middle and upper portion of the crypt (Deschner and Maskens, 1982). On the basis of the hypothesis that abnormal proliferation of crypt cells induced by bile acids is either delayed or normalized by saponins that bind to bile acids.

Saponins extracted from *Agave cantala* and *Asparagus curillus* also significantly inhibited the growth of human cervical carcinoma (JCT-26) and P- 388 leukemia cells (Sati *et al*, 1985). Similarly, the fruit of horse chestnuts has been used to treat mammary cancer (Konoshima and Lee, 1986).

Conclusion

Ethnomedicine has been instrumental in providing important clues as to the role of herbs and foods and their bioactive constituents in disease prevention and therapy; however, experimental based evidence in support of plant-derived notions would lead to the development of products relevant to drug development. Several naturally-occurring compounds such as those in edible plants or spices are known to target multiple molecular pathways of signalling, thus a broad preventive/therapeutic potential against several diseases have been conferred on them. The natural compounds have also been tested in clinical trials as potential therapeutics against several diseases. In the above sub-sections, we have discussed in detail, the anticarcinogenic effect of diosgenin.

Large number of studies have revealed that diosgenin posses therapeutic actions such as anti-inflammatory and anticancer. Diosgenin is reported to stabilize lysosomal membrane and causes uncoupling of oxidative phosphorylation and have strong oxygen radical scavenging activity. Most interesting feature of diosgenin is lack of intestinal side effects, thus it is used in the synthesis of oral contraceptives, sex hormones. More recent work is needed in order to explore its new areas of application.

The main objective of this review was to explore the potential efficacy of saponin (diosgenin), in preventing carcinogenesis in vivo and to understand the anticancer mechanisms of diosgenin in vitro.

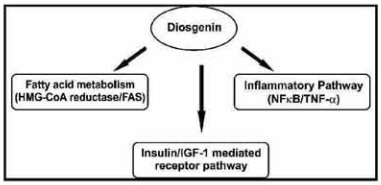


Fig. 7. Possible roles of Diosgenin in signalling studies (Raju and Rao, 2012)

The health promoting effects of diosgenin can be broadly divided according to the differential molecular mechanisms it elicits. First, there is a growing body of experimental evidence suggesting the use of diosgenin in the treatment of metabolic diseases. Much of this is rendered through diosgenin's capacity to lower lipids in the blood and perhaps in tissues such as liver and adipose tissue. Second, the role of diosgenin in modulating cancers has been substantially addressed; most of these data are related to the growth and proliferation of human cancer cell types and its potential mechanism(s) of action in vitro.

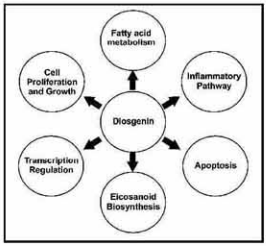


Fig. 8. Schematic representation of mechanism of action(s) of Diosgenin at the cellular level as a cancer chemopreventive/therapeutic agent (Raju and Rao, 2012)

The induction of apoptosis by diosgenin is in part affected by its ability to suppress the expression of the antiapoptotic bcl-2 while increasing the expression of the proapoptotic caspase-3 inducing apoptosis and differentiation, enhancing the immune system, inhibiting angiogenesis and reversing multidrug resistance. However, the mechanism of the anticancer activity has not yet been fully elucidated. Further research is needed to explore the molecular mechanism of herbal drugs. Although the clinical trials showed that herbs were helpful against cancer, these outcomes require further confirmation with strictly controlled trials. Many clinical trials focusing on the anticancer effects of herbal formulas have been conducted. Though many of them demonstrated that herbs are helpful against cancer, especially useful in improving survival and quality of life in patients suffering from advanced cancer, the lack of controls and reporting bias have been posing severe flaws. Researchers must pay attention to the scientific rigor of studies of herbal drugs in the future to improve the status. Some herbs may be harmful to the human body if they are used improperly. Some herbs may cause serious toxicity when taken excessively or under inappropriate circumstances. Also, potential herb-drug interactions should be taken into consideration if multiple drugs are prescribed simultaneously. In future research is required to determine which ingredients are effective and will provide valuable clues for searching and developing anticancer drugs in the future. In addition it is required to determine which ingredients are effective as it will provide valuable clues for researching and developing anticancer drugs in the future, for this saponin. So the proposed outcome of

the study is to select the optimum dose and formulation of a polyherbal drug for therapeutic purpose.

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