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Diosgenin, a Steroid Saponin Constituent of Yams and Fenugreek: Emerging Evidence for Applications in Medicine

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1. Introduction

Phytochemicals found in foods and spices are progressively gaining popularity over conventional synthetic drugs mainly because they act via multiple molecular targets that synergize to efficiently prevent or treat chronic illnesses. Phytochemicals are also safe (with minimal or no toxic or side effects) with better bioavailability. Food saponins have been used in complimentary and traditional medicine against a variety of diseases including several cancers. Diosgenin, a naturally-occurring steroid saponin is found abundantly in legumes (Trigonella sp.) and yams (Dioscorea sp.). Diosgenin is a precursor of various synthetic steroidal drugs that are extensively used in the pharmaceutical industry. Over the past two decades, a series of pre-clinical and mechanistic studies have been independently conducted to understand the beneficial role of diosgenin against metabolic diseases (hypercholesterolemia, dyslipidemia, diabetes and obesity), inflammation and cancer. In experimental models of obesity, diosgenin decreases plasma and hepatic triglycerides and improves glucose homeostasis plausibly by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues. A limited number of experiments have been conducted to understand the pre-clinical efficacy of diosgenin as a chemopreventive/therapeutic agent against cancers of several organ sites. Mechanistic studies using in vitro models suggest that diosgenin suppresses cancer cell growth through multiple cell signaling events associated with proliferation, differentiation, apoptosis, inflammation and oncogenesis. This chapter provides a comprehensive review of the biological activity of diosgenin that contributes to several diseases in its role as a health beneficial phytochemical by citing new studies. In addition, diosgenin’s safety with regards to its potential toxicity is also critically discussed. Altogether, the findings from pre-clinical and mechanistic studies strongly implicate the use of diosgenin as a novel multi-target based chemopreventive or therapeutic agent against several chronic diseases.
1.1 Background of origin

Diosgenin is a major bioactive constituent of various edible pulses and roots, well characterized in the seeds of fenugreek (*Trigonella foenum graecum* Linn) as well as in the root tubers of wild yams (*Dioscorea villosa* Linn) (No authors listed, 2004; Taylor et al., 2000). Reference to the ethnobotanical use of fenugreek seeds appears in the Egyptian Ebers papyrus (c. 1500 BC) as a medicine to induce childbirth. Fenugreek seeds were referred as a “soothing herb” by the Greek physician Hippocrates (5th century BC) and Dioscorides (1st century AD) suggested its use in the treatment of gynaecological inflammation (Chevalier, 2000). Wild yam tubers on the other hand have been used traditionally as a pain reliever by the ancient Aztec and Mayan people in the Americas (Chevalier, 2000). Data available from various traditional medical practices indicate that fenugreek seeds and wild yam tubers have been purported to be used as a preventive or therapeutic medicine against several ailments including arthritis, cancer, diabetes, gastrointestinal disorders, high cholesterol, and inflammation suggesting a variety in its use (Memorial Sloan-Kettering Cancer Center).

1.2 Chemistry, structure-function and bioavailability

Structurally, diosgenin [(25R)-spirost-5-en-3b-ol] is a spirostanol saponin consisting of a hydrophilic sugar moiety linked to a hydrophobic steroid aglycone (Figure 1). Diosgenin is structurally similar to cholesterol and other steroids. Since its discovery, diosgenin is the single main precursor in the manufacture of synthetic steroids in the pharmaceutical industry (Djerassi et al., 1952). In a recent study it was shown that spirostanol compounds, especially diosgenin glycosides exhibited inducible or inhibitory activity in rat uterine contraction based on (a) the number, length and position of sugar side chains attached by a glycoside, and (b) related to the structure of the aglycone (Yu et al., 2010). The structure-related functions of diosgenin have been extensively tested using cancer cells *in vitro*. In comparison to two-structurally-related saponins, hecogenin and tigogenin, only diosgenin caused a cell cycle arrest associated with strong apoptosis *in vitro* (Corbiere et al., 2003). The biological activities of diosgenin and other structurally-related steroid saponins and alkaloids were tested *in vitro* (Trouillas et al., 2005). By using molecular modelling, the spatial conformation and electron transfer capacity were calculated in relation to the structural characteristics of diosgenin necessary to elicit its effect on proliferation rate, cell cycle distribution and apoptosis; and the anti-cancer bioactivity of diosgenin was shown to be related to the presence of a hetero-sugar moiety and the 5,6-double bond in its structure (Trouillas et al., 2005). Moreover, structural conformation at C-5 and C-25 carbon atoms was shown to be important for diosgenin’s biological activity (Trouillas et al., 2005). Further studies are warranted to assess the structure-function relationship of diosgenin and to understand whether and how synthetic changes brought about could augment its biological activity in favour of its role as a therapeutic agent. Rodent studies on the disposition of diosgenin revealed that diosgenin was poorly absorbed and underwent extreme biotransformation (Cayen et al., 1979). In the same study, 1 µg/mL of diosgenin was recovered from the serum of human subjects receiving an oral dose of 3 g diosgenin per day for 4 weeks suggesting poor absorption and possibly active biotranformation (Cayen et al., 1979).
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2. Metabolic diseases

Fenugreek seeds and *Dioscorea* sp. yam tubers together with their constituent diosgenin have been shown to have biological activity against several metabolic diseases (Ulbricht et al., 2007; Raju & Rao, 2009). In the following sections, studies that have used the active constituent diosgenin in both experimental models of metabolic diseases and human clinical studies are reviewed. A list of studies evaluating the effects of diosgenin against different metabolic diseases is summarised in Table 1.

<table>
<thead>
<tr>
<th>Disease/ Effect</th>
<th>Experimental model</th>
<th>Beneficial potency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia/ obesity</td>
<td>Cholesterol-fed rats</td>
<td>Y</td>
<td>Cayen and Dvornik, 1979</td>
</tr>
<tr>
<td>Cholesterol-lowering</td>
<td>Cholesterol-fed chickens</td>
<td>Y</td>
<td>Juarez-Oropeza et al., 1987</td>
</tr>
<tr>
<td></td>
<td>Cholesterol-fed rabbits</td>
<td>Y</td>
<td>Son et al., 2007</td>
</tr>
<tr>
<td>Adipocyte- differentiation/ inflammation</td>
<td>Normal rats</td>
<td>N</td>
<td>Cayen and Dvornik, 1979</td>
</tr>
<tr>
<td></td>
<td>3T3-L1 (Mouse embryonic fibroblast - adipose like cells)</td>
<td>Y</td>
<td>Cayen and Dvornik, 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Uemura et al., 2010</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Streptozotocin-induced diabetic rats</td>
<td>Y</td>
<td>McAnuff et al., 2005</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>FVB mice</td>
<td>Y</td>
<td>Kosters et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Wistar rats</td>
<td>Y</td>
<td>Nibbering et al., 2001</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>NPC1L1-knockout (L1KO) and wild-type mice</td>
<td>Y</td>
<td>Nervi et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temel et al., 2009</td>
</tr>
</tbody>
</table>

Table 1. Effect of diosgenin (purified) on metabolic and related diseases
2.1 Dyslipidemia and obesity

The lipid-lowering potential of diosgenin has been demonstrated by several experimental studies (Sauvaire et al., 2000). Diosgenin decreased the elevated cholesterol in serum LDL and HDL fractions in cholesterol-fed rats, and had no effect on serum cholesterol in normocholesterolemic rats. In addition, diosgenin inhibited cholesterol absorption, and suppressed its uptake in serum and liver, and its accumulation in the liver (Cayen and Dvornik, 1979). Diosgenin lowered plasma cholesterol in diet-induced hypercholesterolemic rats, chicken and rabbits when administered orally or parenterally (Juarez-Orozpeza et al., 1987). Recently, it was shown that diosgenin (at a oral dose of 0.1% or 0.5% in the diet for 6 weeks) decreased total cholesterol levels and increased the plasma high-density lipoprotein (HDL) cholesterol level in both plasma and livers of diet-induced hypercholesterolemic rats (Son et al., 2007). In a study that evaluated the anti-obesity role of diosgenin-rich Dioscorea nipponica Makino, a related species to Dioscorea villosa, Sprague-Dawley rats fed a diet containing 40% beef tallow and 5% freeze-dried extract of the yam gained less body weight and adipose tissue than those that received only the 40% beef tallow diet (Kwon et al., 2003). In the same study it was shown that diosgenin suppressed the time-dependent increase of blood triacylglycerol levels when orally administered with corn oil to ICR mice, suggesting an inhibitory potential against fat absorption (Kwon et al., 2003).

An evidence-based systemic review clearly suggests that fenugreek seeds (rich in diosgenin content) have an important role in the control of metabolic diseases such as diabetes and obesity (Ulbricht et al., 2007). Fenugreek decreased the size of adipocytes in diabetic obese KK-Ay mice suggesting an increased differentiation of adipocytes leading to decreased adipocyte lipid accumulation (Uemura et al., 2010). These results were further validated with molecular data that showed that fenugreek increased the mRNA expression levels of differentiation-related genes in adipose tissues (Uemura et al., 2010). Furthermore, in vitro experiments using 3T3-L1 adipocytes showed that diosgenin, the major saponin in fenugreek, promoted 3T3-L1 adipocyte differentiation to enhance insulin-dependent glucose uptake (Uemura et al., 2010). Two clinical studies were recently published showing the anti-obesity properties of fenugreek seeds. First, a double-blind randomized placebo-controlled three-period (14 days) cross-over trial with twelve healthy male volunteers, demonstrated that fenugreek seed extract selectively reduced spontaneous fat consumption compared to placebo controls (Chevassus et al., 2009). However, there was no effect on body weight, normal and fasting glucose levels, insulin and lipid profile, and visual analogue scale scores of appetite/satiety in subjects receiving the fenugreek seed extract (Chevassus et al., 2009). In the second study, a 6-week double-blind randomized placebo-controlled parallel trial with thirty-nine healthy overweight male volunteers, showed decreased dietary fat consumption in subjects that received a fixed dose of a fenugreek seed extract compared to those that received the placebo (Chevassus et al., 2010). In addition, subjects that received the fenugreek seed extract also demonstrated a decrease in the insulin/glucose ratio in the serum of fasted subjects (Chevassus et al., 2010). Put together, these two clinical studies provided evidence to support that fenugreek seeds may potentially regulate fat consumption in humans. Whether diosgenin per se can mimic these results at appreciable doses in human subjects need to be addressed.
2.2 Diabetes

There are several reports suggesting that diosgenin-rich food sources such as fenugreek seeds and yam tubers contribute to anti-diabetic effects in experimental models (Basch et al., 2003; Omoruyi, 2008). Evidence from human clinical trials clearly suggests that fenugreek seeds improve blood glucose and other metabolic parameters leading to treatment of diabetes (Basch et al., 2003). Diosgenin significantly decreased plasma glucose in streptozotocin-induced diabetic rats by comparison to the diabetic controls suggesting its anti-diabetic properties (McAnuff et al., 2005). These results were further strengthened by the fact that several hepatic rate-limiting enzymes commonly involved in glucose metabolism altered in the diabetic state were normalized by treatment with diosgenin (McAnuff et al., 2005). While there is ample evidence (including clinical trials) suggesting that fenugreek seeds may be used as an alternative medicine to treat diabetes and associated complications, more experimental studies are warranted to address if diosgenin can be efficaciously used in the control of diabetes and to understand the mechanism(s) of action.

2.3 Liver function, liver disease and bile secretion

There is a plethora of information that suggests that both fenugreek seeds and yam tubers influence several metabolic diseases directly affecting a number of molecular targets involved in enzyme metabolism as well as signal transduction pathways in the liver suggesting that their active compounds such as diosgenin may plausibly modulate liver function and may aid in the therapeutic control of liver diseases. A lyophilized fraction of Dioscorea sp. yam tubers attenuated CCl4-induced hepatic fibrosis in rats in a dose-dependent manner (Chan et al., 2010). On the other hand, aqueous extract of fenugreek seeds contributed to a significant histopathological protection against ethanol-induced liver toxicity in rats (Thirunavukkarasu et al., 2003). Furthermore, the authors reported that this protection against ethanol-induced toxicity was by modulating lipid peroxidation and the antioxidant status (Thirunavukkarasu et al., 2003). Powdered fenugreek seeds administered in the diet at a dose of 5% (wt/wt) reduced the liver weight and alleviated hepatic steatosis in Zucker obese (fa/fa) rats (Raju and Bird, 2006). The main mechanism of fenugreek seeds in controlling hepatic steatosis was through lowering plasma tumor necrosis factor (TNF)-α, a proinflammatory cytokine and by decreasing total fat and triglycerides in the liver (Raju and Bird, 2006). Specifically, there are no reports demonstrating the therapeutic potency of diosgenin against liver disease. It is postulated that diosgenin feeding causes cholesterol excretion in the stool of experimental animals, mainly by cholesterol secretion from the bile (Cayen and Dvornik, 1979). Diosgenin has been shown to increase cholesterol secretion five-to seven-fold in the bile of rats without altering the output of bile salts and phospholipids (Kosters et al., 2005; Nervi et al., 1988; Nibbering et al., 2001). Recently, it was shown that the biliary cholesterol secretion stimulated by diosgenin and leading to fecal cholesterol excretion is independent of intestinal cholesterol absorption (Temel et al., 2009).

3. Cancer

3.1 In vivo studies

There are limited experimental studies addressing the in vivo tumor modulating potential of diosgenin (summarised in Table 2). Diosgenin inhibited the formation of colon aberrant crypt foci (ACF), putative precancerous lesions induced by azoxymethane (AOM) in F344...
In this study, administration of diosgenin in the diet at a dose of 0.1% and 0.05% (wt/wt) either during initiation/post-initiation or promotion stages significantly suppressed AOM-induced colon ACF (Raju et al., 2004). The demonstrated ability of diosgenin to inhibit both the total number of ACF and large ACF (those with crypt multiplicity of four or more) suggests that it could effectively prevent, retard and cease the appearance and growth of precancerous lesions in the colon (Raju et al., 2004). Furthermore the lower dose of 0.05% was as effective as the higher dose of 0.1% in blocking ACF formation (Raju et al., 2004). In a double-blind study designed to assess the tumor-modulating potential of diosgenin using the AOM-injected F344 rats, Malisetty et al. (2005) reported that 0.1% of diosgenin suppressed the incidence of both invasive and non-invasive colon adenocarcinomas by up to 60% (Malisetty et al., 2005). In addition, diosgenin decreased colon tumor multiplicity (adenocarcinomas/rat) compared to Controls. In part, these in vivo effects were shown to be related to a lower proliferating cell nuclear antigen (PCNA) index in colon tumors suggesting that diosgenin attenuates tumor cell proliferation (Malisetty et al., 2005).

<table>
<thead>
<tr>
<th>Organ site</th>
<th>Pathological target</th>
<th>Experimental model</th>
<th>Inhibition* Yes (Y) or No (N)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Aberrant crypt foci</td>
<td>AOM-induced rats</td>
<td>Y</td>
<td>Raju et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Tumors</td>
<td>AOM-induced rats</td>
<td>Y</td>
<td>Malisetty et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Ulcers/tumors</td>
<td>AOM/DSS-induced ICR mice</td>
<td>Y</td>
<td>Miyoshi et al., 2011</td>
</tr>
<tr>
<td>Breast</td>
<td>MCF-7 tumor xenografts</td>
<td>Nude (nu/nu) mice</td>
<td>Y</td>
<td>Srinivasan et al., 2009</td>
</tr>
<tr>
<td></td>
<td>MDA 231 tumor xenografts</td>
<td>Nude (nu/nu) mice</td>
<td>Y</td>
<td>Srinivasan et al., 2009</td>
</tr>
<tr>
<td>Lung</td>
<td>LA795 ectopic tumors</td>
<td>T739 mice</td>
<td>Y</td>
<td>Yan et al., 2009</td>
</tr>
</tbody>
</table>

*Inhibition of either (a) tumor incidence [number of tumor-bearing animals], (b) tumor multiplicity [number of tumors per tumor-bearing rats], or (c) tumor size.

Table 2. In vivo anticancer effects of diosgenin (purified).

Diosgenin has been shown to attenuate inflammatory process in relevant animal models. For instance, diosgenin dose-dependently attenuated sub-acute intestinal inflammation and normalized bile secretion in indomethacin-induced intestinal inflammation in rats (Yamada et al., 1997). The role of chronic inflammation on carcinogenesis is vital (Dinarello, 2006); thus the study by Yamada et al. (1997) demonstrating the ability of diosgenin to effectively treat inflammation could be extrapolated to its prospective chemopreventive action against cancers. Recently, the efficacy of diosgenin against AOM/dextran sodium sulphate (DSS)-induced inflammation-associated colon carcinogenesis in ICR mice was reported (Miyoshi N et al., 2011). Diosgenin at doses of 20, 100 and 500 mg/kg (wt/wt) in the diet reduced AOM/DSS induced ulcers to 53%, 46% and 40%, respectively in comparison to control (Miyoshi et al, 2011). While diosgenin did not alter the incidence of colon tumors (adenoma + adenocarcinoma), it reduced the tumor multiplicity significantly at all the three tested doses (Miyoshi N et al., 2011). Furthermore, it was shown that diosgenin’s potency against
experimentally-induced inflammation-associated colon carcinogenesis was in part mediated by the alteration of lipid metabolism (reduced serum triglyceride levels by up-regulation of lipoprotein lipase), and the modulation of genes associated with inflammation and multiple signaling pathways (Miyoshi N et al., 2011).

With regards to breast cancer, the effect of diosgenin on the ectopic growth of human breast cancer MCF-7 and MDA 231 tumor xenografts was studied in nude mice (Srinivasan et al., 2009). It was reported that diosgenin (10 mg/kg body weight administered intra-tumorally) significantly inhibited the growth of tumor xenografts of both MCF-7 and MDA 231 compared to vehicle-treated controls, with no toxicity to any of the vital organs in the experimental mice (Srinivasan et al., 2009). To test the anti-aging properties of diosgenin in relation to hormonal-effects in vivo, Tada et al. (2009) assessed the effect of diosgenin-rich Dioscorea Sp. yam tuber extract on the ectopic growth of estradiol-dependent human breast cancer (MCF-7) in ovariectomized nude mice for 12 weeks. Diosgenin containing extracts was shown to repress the size of the tumors compared to sham controls (Tada et al., 2009). Yan et al. (2009) reported that oral administration of diosgenin at a dose of 200 ppm (p.o.) significantly inhibited the growth mouse LA795 lung adenocarcinoma tumors by 33.94% in T739 inbred mice.

3.2 In vitro studies

The anti-cancer effects of diosgenin in vitro through different mechanisms are discussed in the following sub-sections. Many molecular candidates critical to tumorigenesis are affected by diosgenin (Raju and Mehta, 2009; Raju and Rao, 2009). The in vitro anticancer effects of diosgenin and its cellular/molecular effects is summarised in Table 3.

3.2.1 Colon cancer

Diosgenin inhibited the growth of HT-29 and HCT-116 human colon adenocarcinoma cells (Lepage et al., 2010; Raju and Bird, 2007; Raju et al., 2004). Diosgenin induced apoptosis in HT-29 cells, in part by inhibition of bcl-2 and by induction of caspase-3 protein expression (Raju et al., 2004). Lepage et al. (2010) reported that in HT-29 cells, diosgenin at 40 µM caused delayed apoptosis together with an increase in cyclooxygenase (COX)-2 expression and activity, higher 5-lipoxygenase (LOX) expression and enhanced leukotriene B4 production. COX-2 inhibition by NS-398 strongly sensitized HT-29 cells to diosgenin-induced apoptosis (Lepage et al., 2010). Furthermore, diosgenin was shown to sensitize HT-29 cells to TRAIL-induced apoptosis (Lepage et al., 2011). In HCT-116 cells, diosgenin was shown to induce apoptosis by the cleavage of the 116 kDa poly (ADP-ribose) polymerase (PARP) protein to the 85kDa fragment (Raju and Bird, 2007). In addition, it was shown that diosgenin significantly lowered the expression of HMG-CoA reductase at both mRNA and protein levels, suggesting the involvement of the cholesterol biosynthetic pathway in diosgenin’s efficacy as an anti-cancer agent (Raju and Bird, 2007).

3.2.2 Breast cancer

Diosgenin arrested the growth of HER2 oncoprotein-overexpressing AU565 human breast adenocarcinoma cells at sub-G1 phase (Chiang et al., 2007). Selective apoptosis induced by diosgenin in these cells was found to be through PARP cleavage involving the down-
<table>
<thead>
<tr>
<th>Organ site</th>
<th>Cancer cell type</th>
<th>Cellular/molecular targets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>HT-29</td>
<td>Apoptosis/ bcl-2, caspase-3, COX-2, 5-LOX</td>
<td>Raju et al., 2004; Lepage et al., 2010; 2011</td>
</tr>
<tr>
<td></td>
<td>HCT-116</td>
<td>Apoptosis/ PARP, COX-2, 5-LOX</td>
<td>Raju et al., 2007; Lepage et al., 2010; 2011; Raju et al., 2007</td>
</tr>
<tr>
<td>Breast</td>
<td>AU565</td>
<td>Apoptosis/ PARP, mTOR, JNK Lipid metabolism/FAS</td>
<td>Chiang et al., 2007; Srinivasan et al., 2009</td>
</tr>
<tr>
<td></td>
<td>MCF-7</td>
<td>Growth-proliferation/ Akt, p53 Apoptosis/ NF-κB, Bcl-2, survivin</td>
<td>Srinivasan et al., 2009; Srinivasan et al., 2009</td>
</tr>
<tr>
<td></td>
<td>MDA 231</td>
<td>Growth-proliferation/ ERK Apoptosis/caspase-3, NF-κB, Bcl-2, survivin</td>
<td>Srinivasan et al., 2009; Srinivasan et al., 2009</td>
</tr>
<tr>
<td>Prostate</td>
<td>PC-3</td>
<td>Growth-proliferation/ PI3K, Akt, ERK, JNK, NF-κB Angiogenesis/ VEGF</td>
<td>Chen et al., 2011</td>
</tr>
<tr>
<td></td>
<td>DU145</td>
<td>Growth-invasion/ HGF, mdm2, vimentin, Akt, mTOR</td>
<td>Chang et al., 2011</td>
</tr>
<tr>
<td>Cervix</td>
<td>CaSki</td>
<td>Growth-proliferation</td>
<td>Fernández-Herrera et al., 2010</td>
</tr>
<tr>
<td>Liver</td>
<td>HCC</td>
<td>Apoptosis/caspase-3, PARP Transcription/ STAT3, cSrc</td>
<td>Li et al., 2010</td>
</tr>
<tr>
<td>Bone/blood</td>
<td>1547 osteosarcoma</td>
<td>Apoptosis/ NF-κB, p53, PPAR-γ, COX-2</td>
<td>Corbiere et al., 2003; 2004a; Moalic et al., 2001</td>
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<tr>
<td></td>
<td>RAW-264.7</td>
<td>Growth-proliferation/ NF-κB, RANK-L</td>
<td>Shishodia and Aggarwal, 2006</td>
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<tr>
<td></td>
<td>KBM-5</td>
<td>Growth-proliferation/ NF-κB, IκBa, cyclin-D1,</td>
<td>Shishodia and Aggarwal, 2006</td>
</tr>
<tr>
<td></td>
<td>HEL</td>
<td>Apoptosis/ PARP, caspase-3, p21 Eicosanoid biosynthesis/ COX-2, 5-LOX/cPLA2</td>
<td>Leger et al., 2004a; 2006; Leger et al., 2004b; Napez et al., 1995</td>
</tr>
<tr>
<td></td>
<td>K562</td>
<td>Apoptosis/ PARP, caspase-3, NF-κB, COX-2, p38 MAPK</td>
<td>Liagre et al., 2005</td>
</tr>
<tr>
<td>Larynx</td>
<td>HEP-2</td>
<td>Apoptosis/ p53</td>
<td>Corbiere et al., 2004b</td>
</tr>
<tr>
<td>Skin</td>
<td>M4Beu</td>
<td>Apoptosis/ p53</td>
<td>Corbiere et al., 2004b</td>
</tr>
</tbody>
</table>

Table 3. *In vitro* anticancer activities of diosgenin (purified)
regulation of phospho-Akt and phospho- mammalian target of rapamycin (mTOR), and up-regulation of phospho- c-Jun N-terminal kinase (JNK) independent of p38 and extracellular signal regulating kinase (ERK) phosphorylations (Chiang et al., 2007). In the same cell-line (AU565 cells), diosgenin inhibited the expression of fatty acid synthase (Chiang et al., 2007). The anti-cancer mechanism of diosgenin was shown to be different in human breast cancer cells based on the status of estrogen receptor (ER) expression (Srinivasan et al., 2009). In ER-positive MCF-7 human breast cancer cells, diosgenin induced p53 tumor suppressor protein; while the pro-apoptotic mechanism of diosgenin in ER-negative MDA human breast carcinoma cells involved the activation of caspase-3 and down-regulation of bcl-2 (Srinivasan et al., 2009).

3.2.3 Prostate cancer

Diosgenin inhibited proliferation of PC-3 human prostate cancer cells in a dose-dependent manner (Chen et al., 2011). At non-toxic doses, diosgenin suppressed cell migration and invasion by reducing the activities and mRNA expression of matrix metalloproteinase (MMP)-2 and MMP-9 (Chen et al., 2011). Diosgenin abolished the expression of vascular endothelial growth factor (VEGF) in PC-3 cells and tube formation of endothelial cells (Chen et al., 2011). In addition, diosgenin downregulated the phosphorylation of phosphatidylinositide-3 kinase (PI3K), Akt, ERK and JNK proteins and significantly decreased the nuclear level of nuclear factor (NF)-κB, suggesting that diosgenin inhibited NF-κB activity (Chen et al., 2011). Another study using DU145 human prostate cancer cells found that diosgenin abrogated hepatocyte growth factor (HGF)-induced cell scattering and invasion, together with inhibition of Mdm2 and vimentin through down-regulation of phosphorylated Akt and mTOR (Chang et al., 2011).

3.2.4 Cervical cancer

Recently, Fernández-Herrera et al. (2010) reported the synthesis of a novel 26-hydroxy-22-oxocholestanic steroid from diosgenin and its anticancer activity against human cervical cancer CaSki cells. Mainly this diosgenin-derivative caused apoptosis at non-cytotoxic doses activation of caspase-3 along. Furthermore, they report that antiproliferative doses of this compound observed in cancer cells did not affect the proliferative potential of normal fibroblasts from cervix and peripheral blood lymphocytes (Fernández-Herrera et al., 2010).

3.2.5 Liver cancer

Diosgenin was shown to inhibit the proliferation of hepatocellular carcinoma (HCC) cells in a dose and time-dependent manner (Liu et al., 2005). Diosgenin caused arrest of HCC cells at the G1 phase of the cell cycle and induced apoptosis through caspase-3 activation leading to PARP cleavage (Li et al., 2010). In these cells, diosgenin inhibited both constitutive and inducible activation of signal transducers and activators of transcription (STAT)3 with no effect on STAT5, and suppressed the activation of c-Src, Janus-family tyrosine kinases (JAK)1 and JAK2 implicated in STAT3 activation (Li et al., 2010).

3.2.6 Other cancers

Cytotoxic effects of diosgenin were reported in human cancer cell lines of various other organ types: osteosarcoma (Corbiere et al., 2003, Moalic et al., 2001), leukemia (Liu et al., 2005).
2005) and erythroleukemia (Leger et al., 2004a). The growth of 1547 human osteosarcoma cells was inhibited through G₁ phase cell cycle arrest and induction of apoptotic demise; and the main mechanism involved the activation of p53 and binding of NFκB to DNA independent of PPAR-γ (Corbiere et al., 2003, 2004a). Interestingly, diosgenin’s effect in suppressing osteoclastogenesis in RAW-264.7 cells was reported to follow a pro-apoptotic mechanism through receptor activated NFκB ligand (RANK-L) induction (Shishodia and Aggarwal, 2006). Moalic et al. (2001) demonstrated that diosgenin inhibited COX-2 activity and expression in human osteosarcoma 1547 cells.

Diosgenin arrested chronic myelogenous leukemia KBM-5 cells of human origin at sub-G₁ phase of cell cycle arrest (Shishodia and Aggarwal, 2006). This cell cycle arrest was correlated to the inhibition of tumor necrosis factor (TNF)-dependent NF-κB activation and TNF-induced degradation and phosphorylation of IκBα (the inhibitory subunit of NF-κB) (Shishodia and Aggarwal, 2006). Diosgenin downregulated TNF-induced cyclin D1 in KBM-5 cells (Shishodia and Aggarwal, 2006). Furthermore it abolished both basal and TNF-induced COX-2 gene products in a time-dependent manner (Shishodia and Aggarwal, 2006). Diosgenin controlled the growth of human erythroleukemia TIB-180 (HEL) cells by arresting cells at G₂/M cell cycle and inducing apoptosis through PARP cleavage, activation of caspase-3 and up-regulation of p53-independent p21 (Leger et al., 2004a, 2004b, 2006). Diosgenin treatment to HEL cells induced cytosolic phospholipase (cPL)A2 activation through translocation to the cellular membrane together with an increase in COX-2 expression (Leger 2004b). In a study by Nappez et al. (1995), it was reported that diosgenin treatment in HEL cells did not affect 5-LOX mRNA or 5-LOX activating protein (FLAP) mRNA at the transcriptional level. However, when HEL cells undergoing differentiation were incubated with diosgenin in the presence of indomethacin (a COX inhibitor), the growth inhibitory effect of diosgenin was reversed and an exponential growth kinetic of undifferentiated cells was observed (Nappez et al., 1995). Taken together, these studies provide an insight into the role of 5-LOX in diosgenin’s modulation of growth and differentiation in HEL cells. Leger et al. (2004b) demonstrated that diosgenin increased the synthesis of arachidonic acid in HEL cells leading to COX-2 overexpression, which was accompanied by apoptosis induction. The anticancer activity of diosgenin was also shown in human erythroleukemia K562 cells through caspase-3-activation dependent PARP-mediated pro-apoptotic effects (Liagre et al., 2005). Diosgenin induced COX-2-independent apoptosis through activation of the p38 MAP kinase signalling pathway and inhibition of NFκB binding in COX-2 deficient K562 cells (Liagre et al., 2005). The anticancer mechanism of diosgenin was also shown in laryngocarcinoma HEp-2 and melanoma M4Beu cells through a p53-dependent cell death mechanism (Corbiere et al., 2004b).

4. Other diseases and ailments

A clinical study by Turchan-Cholewo et al. (2006) reported that diosgenin may have therapeutic potential against an increased risk of developing dementia in opiate abusers with HIV infection. Recently, the effect of diosgenin on hepatitis C virus (HCV) replication was reported (Wang et al., 2011). Based on a reporter-based HCV subgenomic replicon system, diosgenin was found to inhibit HCV replication at low µM concentrations in vitro (Wang et al., 2011). Furthermore, a combination of diosgenin and interferon-α exerted an additive effect on the resultant anti-HCV activity (Wang et al., 2011). The neuroprotective
effect of diosgenin against d-galactose-induced senescence in mice by improving cognitive abilities as assessed through the Morris water maze test and mediated by enhancing endogenous antioxidant enzyme activities (Chiu et al., 2011). Diosgenin induced apoptosis in non-cancerous human rheumatoid arthritis synoviocytes (RAS) through the overexpression of COX-2 protein and concomitant increase in the level of prostaglandin (PG)-E$_2$ (Liagre et al., 2004, 2007). Diosgenin may be an effective inhibitor of hyperpigmentation, commonly seen in skin disorders and inhibits melanogenesis by activating the PI3K pathway and (Lee et al., 2007). A novel effect of diosgenin in restoration of keratinocyte proliferation in aged skin in an animal model suggests that diosgenin may have a potential in slowing the aging process in the skin commonly associated with climacteric (Tada et al., 2009). Recent data support the possibility that some diosgenin-glycoside derivatives may represent a new type of contractile agonist for the uterus and their synergism may be responsible for their therapeutic effect against abnormal uterine bleeding (Yu et al., 2010).

5. Safety

There is a claim that diosgenin has an endocrine effect (estrogen-like or progesterone-like activity) in humans, however there is no scientific evidence to its validation (Djerassi et al., 1952; No authors listed, 2004). Diosgenin is neither synthesized nor metabolically converted into steroid by-products in the mammalian body. Toxicology studies using relevant experimental models have established that even at an upper concentration of 3.5% (wt/wt), diosgenin was safe and failed to cause systemic toxicity, genotoxicity, or estrogenic activity (63). Qin et al. (2009) reported that ethanol extracts of Dioscorea sp. containing 28.34% (wt/wt of lyophilized powder) did not cause any signs of acute toxicity in mice at an upper tested dose of 562.5 mg/kg/d, and did not significantly change toxicological parameters up to a dose of 255 mg/kg/d. No acute renal or hepatic toxicity associated with the administration of extracts of Dioscorea villosa at an oral dose of 0.79 g/kg/d (Wojcikowski et al., 2008). However, an increase in fibrosis in the kidneys and in inflammation in livers when rats were on the dose for 28 days was reported in the same study (Wojcikowski et al., 2008). Thus it was concluded that these toxicology effects be considered when consuming these extracts on a long-term basis, especially in people with compromised renal or hepatic function (Wojcikowski et al., 2008). On the other hand, in a 90-day subchronic study, rats fed fenugreek seeds, at doses between 1% and 10% in pure diet, had no toxic effects (Muralidhara et al., 1999). Typically, fenugreek seeds are known to contain ~0.42% to 0.75% diosgenin depending on the cultivars and seed quality (Taylor et al., 2000). Fenugreek seed extract evaluated using the standard battery of tests (reverse mutation assay, mouse lymphoma forward mutation assay and mouse micronucleus assay) recommended by US Food and Drug Administration (FDA) for food ingredients came negative and thus rendered safe for consumption at the therapeutic doses tested (Flammang et al., 2004). A recent study using human sera indicated that fenugreek seed powder contains several proteins that potentially act as allergens (Faeste et al., 2009); however, it remains unclear if diosgenin in interaction with any proteins would test positive in these allergen tests. More studies are warranted to understand the toxicological effects of diosgenin at both levels present in common foods as well as therapeutic doses. Although there is toxicology data with regards to diosgenin-rich botanicals such as Dioscorea sp. yam tubers and fenugreek seeds, there is a clear lack of data studying the safety of diosgenin per se. Some of the important aspects for
future studies assessing the toxicology of diosgenin include those related to developmental toxicity, neurotoxicity and allergenicity.

6. Conclusions

With changing lifestyle patterns such as diet and physical activity combined with factors such as genetic predispositions and smoking, the incidences of metabolic diseases including diabetes and obesity and certain kinds of cancers are increasing worldwide and hence are a public health concern with major economic impacts. While the pathogenesis of these diseases is different, there appears to be one or more molecular candidates that are commonly up- or down-regulated leading to the notion that these could be common molecular targets in prevention or therapeutic interventions of diseases. Ethnomedicine has been instrumental in providing important clues as to the role herbs and foods and their bioactive constituents in disease prevention and therapy; however, rigorous experimental-based evidence in support of ethnomedicine-derived notions would lead to the development of products relevant to and drug development. Several naturally-occurring compounds such as those in edible plants or spices are known to target multiple molecular pathways of signalling, thus bestowing them a broad preventive/therapeutic potential against several diseases. For example, curcumin from turmeric, resveratrol from grapes and epigallocatechin gallate (EGCG) from green tea have shown excellent pre-clinical potency against a wide range of diseases (reviewed in Epstein et al., 2010; Marques et al., 2009; Saito et al., 2009). These natural compounds have also been tested in clinical trials as potential therapeutics against several diseases. One emerging natural compound of interest with similar potency as curcumin, resveratrol and EGCG is diosgenin. In the above sub-sections, we have discussed in detail, the health promoting effects of diosgenin and diosgenin-rich

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**Fig. 2.** Schematic representation depicting the molecular mode of action of diosgenin in the control of metabolic pathway. Diosgenin plausibly regulates signaling molecules in fatty acid metabolism and inflammatory pathway. Insulin and IGF-1 mediated signalling pathways may also be regulated by diosgenin and are thus candidates for future studies.
sources: (a) *Dioscorea* sp. yam tubers and (b) *Trigonella* sp. (fenugreek) seeds. In addition, we have summarised the toxicology data pertaining to the safe use of diosgenin either in a pure form (where data was available) or in extracts. 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. To date, there is some evidence that implicates both inflammatory pathway-associated NFκB and fatty acid metabolism-associated HMG-CoA reductase and FAS as potential molecular targets of diosgenin (Chiang et al., 2007; Raju and Bird, 2007), and these may be extended to its role as a therapeutic against metabolic diseases (Figure 2). Pathways associated with insulin and insulin-like growth factor (IGF)-1 may be of relevance in understanding the molecular mechanism of diosgenin’s action in the control of metabolic diseases (Figure 2). 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. Several molecular candidates associated with fatty acid metabolism (Chiang et al., 2007; Raju and Bird, 2007), inflammatory pathway (Leger et al., 2006; Liagre et al., 2005; Shishodia and Aggarwal, 2006), eicosanoid biosynthesis (Leger et al., 2004; Lepage et al., 2010; 2011; Moalic et al., 2001; Napez et al., 1995), cell proliferation and growth (Chiang et al., 2007; Leger et al., 2006; Srinivasan et al., 2009), apoptosis (Corbiere et al., 2004; Leger et al., 2006; Lepage et al., 2010; 2011; Raju and Bird, 2007; Raju et al., 2004), and regulation of transcription (Chiang et al., 2007; Li et al., 2010) are affected (up- or down-regulated) by diosgenin leading to tumor cell death (Figure 3).

![Fig. 3. Schematic representation of plausible mechanism of action(s) of diosgenin at the cellular level as a cancer chemopreventive/therapeutic agent. Diosgenin up- or down-regulates several molecular candidates associated with cell proliferation and growth, apoptosis, regulation of transcription, fatty acid metabolism, inflammatory pathway and eicosanoid biosynthesis leading to tumor cell death.](www.intechopen.com)
While there are ample *in vivo* studies available to implicate the beneficial effects of diosgenin against metabolic disease, more studies to understand the specific modes of action at the molecular level are warranted. On the contrary, substantial *in vitro* evidence exists to understand the molecular mechanism of action of diosgenin against several cancers. More *in vivo* studies are thus essential to understand the physiological relevance of such data in controlling cancers. Moreover, there is excellent opportunity to address whether diosgenin plays a role in chemoprevention versus therapy, or both, in cancers of various organ sites using relevant models. The health beneficial effects of diosgenin are further extended to its potential role to treat other ailments such as HIV and hepatitis-C infections as well as liver diseases. There is little information regarding the bioavailability, pharmacokinetics and pharmacodynamics of diosgenin in relation to its health beneficial effects. Diosgenin and diosgenin-containing products are emerging in the market and are being promoted as natural health products. The scientific knowledge in this area is limited and hence extensive pre-clinical and clinical research should be carried out prior to advocating the safe and efficacious use of diosgenin and diosgenin-rich plant extracts against the prevention and control of diseases. Furthermore, such research will assist in the development of evidence-based regulation of diosgenin and diosgenin-containing products as they become increasingly popular and enter the market.

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9. References
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There are significant concerns regarding the potential side effects from the chronic use of conventional drugs such as corticosteroids, especially in children. Herbal therapy is less expensive, more readily available, and increasingly becoming common practice all over the world. Such practices have both their benefits and risks. However, herbal self-therapy might have serious health consequences due to incorrect self-diagnosis, inappropriate choice of herbal remedy or adulterated herbal product. In addition, absence of clinical trials and other traditional safety mechanisms before medicines are introduced to the wider market results in questionable safe dosage ranges which may produce adverse and unexpected outcomes. Therefore, the use of herbal remedies requires sufficient knowledge about the efficacy, safety and proper use of such products. Hence, it is necessary to have baseline data regarding the use of herbal remedies and to educate future health professionals about various aspects of herbal remedies.

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