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SHORT REVIEW

# Diosgenin as a Novel Therapeutic Natural Product for Various Diseases: An Overview

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## Abstract

Diosgenin (DG) is a saponin glycoside and an active constituent generally present in medicinal plants like *Rhizoma polgonita, Trigonella foenum*, *Dioscorea villosa and Dioscorea rhizome*. It is also the starting material for the biosynthesis of steroidal hormones. DG has also been reported as a multipurpose drug aiding in the treatment of various cardiovascular, neurological diseases as well as as malignancies, osteoporosis, diabetes, and atherosclerosis. The underlying mechanisms of DG contributing towards potential therapeutic ability is to suppress the expression of oncogenic genes, preventing the formation of free radicals and thereby resulting in neuroprotection etc. However, the use of DG is restricted due to its limited pharmacokinetic properties such as poor aqueous solubility, poor bioavailability, and quicker biotransformation into its metabolites. Hence, in this study we have briefed the current therapeutic approaches of DG and its derivatives alongside to its medicinal chemistry and its physicochemical, pharmacological & toxicological properties.

**Keywords:** Biosynthesis, Diosgenin, Phytochemical, Saponin Glycoside, Steroidal Hormone

## 1. Introduction

Diosgenin (DG) is a steroidal saponin usually found in Mexican yams and is a starting precursor for biosynthesis of steroidal hormones. The active constituent of DG is found in herbs; *Rhizoma polgonita, Trigonella foenum*, *Dioscorea villosa and Dioscorea rhizome*[1]. Several studies have proven that isolated form of DG can improve cognitive performance by enhancing the neuronal networks and its morphological activities which can be used in the treatment of various neurodegenerative conditions like Alzheimer's Disease[2]. In recent times, DG is also being studied extensively to prevent and treat malignancies, osteoporosis, cardiovascular disease, diabetes, and atherosclerosis. Due to its high affinity towards the chemokine receptor 3 (CXCR3), which is important in the control of inflammatory responses[3]. Some investigations have revealed DG's therapeutic effects against inflammation and apoptosis mediated via inhibition of Nuclear Factor kappa-B (NF-κB) pathway[4], another study conducted by Li *et al*., found that DG inhibits the expression of NF-κB/Toll-like receptor-4 (TLR4) in lipopolysaccharide induced rat model of Parkinson's disease; thereby attenuating the inflammation and oxidative stress *in vivo* and *in vitro*[5]. DG therapy also reduced the amyloid plaques and Neurofibrillary Tangles (NFTs) load in the cerebral mantle and the hippocampal region of 5XFAD mice via binding to the steroidal binding site protein 1,25D3 -MARRS[6]. Further, DG significantly enhanced neuronal Bcl-2 family-related survival pathways in the D-galactose-induced ageing brain through inhibiting apoptotic signaling pathways[7] and DG diminish the amount of proinflammatory cytokines in Imiquimod (IMQ)-induced psoriasis mice model by downregulating the TLR4/Myd88 receptor[8]. It has been proven that the extract of *Dioscorea zingiberensis* shown anti-thrombotic properties in both *in-vitro* and *in-vivo* thrombotic models[9] and exhibited promising antioxidant activity via cGMP

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route in doxorubicin-induced cardiomyopathy animal models[10]. Recent *in-vivo* investigations suggested that the antioxidant property of DG attenuates hypertension and fibrotic alterations in the heart via cardiac remodeling[11]. According to earlier studies DG inhibiting Neural Precursor Cell Expressed Developmentally Down-Regulated protein-4 (NEDD4) and there by lowers the risk of prostate cancer[12]. Hepatocellular cell growth was also significantly inhibited in HepG2, SMMC-7721, and HCC cell lines for anti-tumorigenic activity in hepatocellular carcinoma[13]. On other hand DG substantially reduced Akt phosphorylation, inhibited the mTOR pathway and enhances the phosphorylation of JNK signalling in breast cancer cell lines, as well as suppresses HER-2 expression[14].

Despite its multiple pharmacological effects, its medicinal use has hindered due to its poor solubility in aqueous medium, bioavailability, pharmacokinetics, and quick biotransformation[15]. This review gives an outline of the sources and the chemistry of DG with its physicochemical, pharmacological and toxicological properties as well as the current therapeutic approaches of DG and its derivatives.

## 2. Sources of Diosgenin

DG is an aglycone generally found in a variety of plants; *Dioscorea rhizome*, *Rhizoma polgonita, Dioscorea zingiberensis, Rhizoma polgonita, Dioscorea villosa, Dioscorea alata, Smilax china* and *Trigonella foenum*[16]. Compared to other species, *D. villosa* and *D. zingiberensis* contain >1% of Diosgenin[17]. *Trillium govanianum* and *Cheilocostus speciosus* contains >2.5% of DG in their extracts[18]. Saponins are, a bioactive phytochemical that has employed as a precursor in the production of steroidal medicines. Generally, Dioscorea tubers used to hydrolysis of in presence of a hydrocarbon solvent and petroleum ether for the isolation of DG[19]. Furthermore, because of its specificity, eco-friendly nature and availability, DG is also being manufactured through microbial transformation technology[20].

## 3. Chemistry

Molecular structure of DG as shown in Figure 1 is composed of 27 C-atoms arranged in 6 rings, 4 six-membered cyclohexane rings fused with 2 five-membered cyclopentane rings and it is substituted by Hydroxyl group (OH) in the 3β position, which carry double bond at position 5-6 in addition to the R substituted configuration at position 25[21]. The molecular formula of Diosgenin is $C_{27}H_{42}O_3$ and has a relative molecular weight of 414.62 g/mol. It is a white colored crystalline powder with a melting point of 204° C-207° C[21,22]. DG is insoluble in water because of its high lipophilicity (with log p of 5.93). However, DG is extremely soluble in organic solvents like Ethyl Acetate (EtOAc), 1,2- dichloroethane ($C_2H_4Cl_2$), Chloroform ($CHCl_3$), Isopropyl Alcohol ($C_3H_8O$) and Ethyl Propionate ($C_5H_{10}O_2$), sparingly soluble in polar solvents like Methanol ($CH_3OH$) and Acetone $(CH_3)_2CO$[1]. In order to synthesize the steroidal hormones, E and F rings of DG (C-16, C-17) is degraded by using 16-Dehydropregnenolone acetate[22,23]. Further, active metabolites of DG are formed by mixing glycoside at 3β-position to generate its saponins like dioscin, which possess various potential bioactive properties; anti-inflammatory, antimicrobial and hypolipidemic properties[24–26].

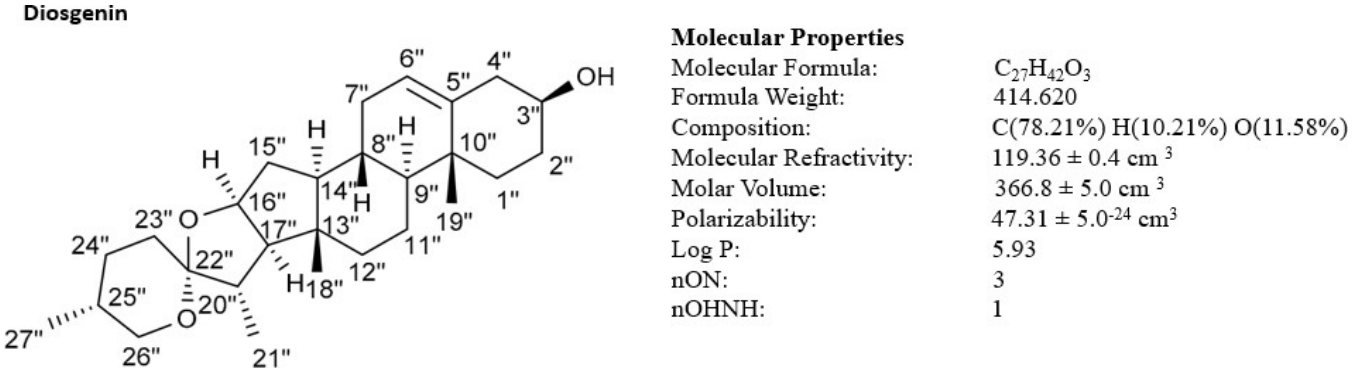


**Figure 1.** Molecular structure and properties of Diosgenin.

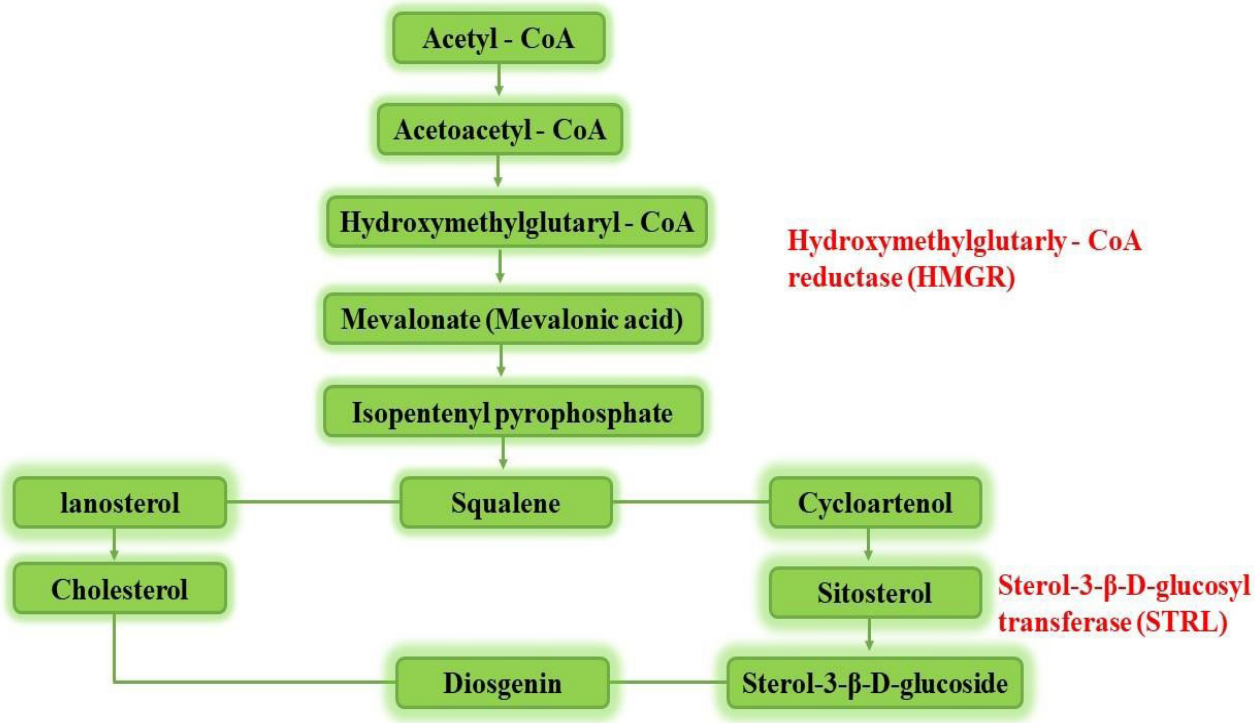


**Figure 2.** Biosynthesis of diosgenin.

## 4. Biosynthesis

DG is produced from squalene-2,3-oxide by two different methods upon treating with *Trigonella foenum* (fenugreek) and methyl jasmonate ultimately generating lanosterol as well as cycloartenol. The starting material acetyl CoA will undergo through a series of processes to create squalene, which is further transformed into lanosterol and cycloartenol as shown in Figure 2[1,27]. Saponins are mono-, di- or tri- saccharides that could convert into DG aglycone to glycoside derivatives[27]. During the process of biosynthesis of DG, cholesterol converts into DG, in general it would be regulated by two CYP-450 enzymes; C-16,22 di- hydroxylase and C-26 hydroxylase[28,29]. Similarly, recent study about Genome sequencing method using CYP-450 gene not only revealed the *de novo* biosynthesis pathway of DG, it also suggested about the new biological pathway for the production of DG from cholesterol[29].

## 5. Pharmacokinetics of Diosgenin

Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) studies revealed that DG has poor bioavailability due to its insoluble nature, poor absorption and metabolic instability in gastric fluids and, in Caco-2 cell line model cyclodextrin complex form of DG showed a better bioavailability than the DG and Dioscin[30]. Studies have also suggested that the bioavailability of DG was found to be very low after oral administration due to its poor solubility. Aqueous solubility of DG was found to be 0.95μg/mL[31]. This limited range of solubility of DG in water might be to reason for its attenuated oral bioavailability. Although an increased oral bioavailability was observed (>45%) in β-cyclodextrin (β-CD) and liquid crystals of DG complex. DG complex reaches its peak concentration with a $C_{max}$ of 132:5 ± 48:2 ng/mL and with an absolute oral bioavailability of 4:45 ± 1:46 %[1,32]. Human Liver microsome (HLM) and S9 fraction metabolic stability assay revealed that DG may be metabolized by enzymes such as CYP 1A2, 2C9, 2D6 and 3A4. Diosgenin and dioscin was found to inhibit CYP3A4 enzyme's catalytic activity, and the $IC_{50}$ values obtained were 9 g/mL (17 M) and 29 g/mL (33 M), respectively[32]. The pharmacokinetic profile of DG in rabbit was found to be $C_{max}$: 0.48, 0.057 mg/ml; $t_{max}$: 1,2 h; and t1/2β: 6.23,15.04 h[33]. However, in animal models; rat model following IV administrations, the concentration-time profiles were best matched to a two-compartment model with good linearity, but at the same concentration time profile of DG in the plasma were not fully described after oral administration with a dose of

22.5 mg/kg though, profiles were highly corresponded to a one-compartment model after administering high oral dosages of 45 and 90 mg/kg. Simultaneously, using 20% v/v organic acid TCA (Trichloroacetic Acid) as a solvent further increases the oral bioavailability of DG[32,34]. These study reports clearly indicates that DG has poor GI absorption until it complexes with β-cyclodextrin or is solubilized with suitable organic solvents. While, an increase in the concentration of DG also decreases the half-life and Clearance (CL).

## 6. Therapeutic Activities

### 6.1 Anti-Inflammatory Activity

DG inhibits the stimulation of NF-Kβ p65/p50, p38, and iNOS production in LPS-treated THP1 cells and in mouse models, according to Gao *et al*[35]. Their findings suggested that DG could be effective in the treatment of Acute Lung Inflammation (ALI)[35]. In animal models of LPS-induced sepsis, oral treatment of DG analogue 15 [(E) 26-(3',4'5'- trimethoxybenzylidene)-furost-5en-3β-acetate] before LPS administration suggestively (P 0.05) reduced production of TNF-α and IL-6. IL-1 production in serum was inhibited (P 0.05). In sepsis analogue 15 also helped to reduce lung and liver damage. It also aided in the survival of mice suffering from fatal sepsis[33]. Similarly, Li *et al*., found that DG reduced LPS-induced Parkinson's Disease (PD) like motor impairments in *in-vitro* and *in-vivo* models, also reducing inflammation and oxidative stress mediating through Inhibition of the TLR/NF-κB signaling pathway may be responsible for these effects[5]. Cai *et al*., conducted research has shown DGP (3,25R)-spirost-2-aminoethyl) amino) ethyl) amino) ethyl) carbamate, a new synthetic DG analogue containing 10 amines significantly reduced the expression of multiple inflammatory mediators in LPS-stimulated microglial BV2 cells which includes (NO) Nitric oxide, (iNOS) Nitric oxide synthase, (TNF-alpha) Tumor Necrosis Factor- alpha, (IL-1), Interleukin-6 (IL-6), and Cyclooxygenase – 2 (COX-2). DGP's anti-inflammatory properties were also accomplished through scavenging ROS and suppressing NF-κB[36].

### 6.2 Alzheimer Disease (AD)

Alzheimer's is the most prevalent progressive neurodegenerative disease with the hallmark symptoms of cognitive impairment and dementia. AD, being a multifaceted progressive disease includes Aβ plagues, neurofibrillary tangles along with Tau protein phosphorylation[37]. According to Tohda *et al*., DG reduced the Aβ plagues by directly stimulating the axonal cell growth and by acting on rapid response steroidal binding receptor (1,25D3-MARRS receptor) in 5FXAD mice model. Also, *in silico* docking scores revealed that DG has more potential binding affinity on 1,25D3-MARRS receptor than the 1,25 Dihydroxyvitamin-D3 therefore, DG possesses more binding affinity than the exogeneous ligand subsequent improvement in the axonal damage[2]. In addition, similar 5FXAD mice model shown improvement in firing of neuron in the prefrontal and hippocampal CA1 region of brain due to increase in the c-Fos expression leads to cognitive enhancement[6]. Lecanu *et al*., reported that caspropinal, a DG derivative improves cognitive function in AD animal model. Similarly, 1,25D3-MARRS receptor activation leads to optimization of the HSC70 by inhibiting the expression of α-tubulin, this might be a crucial step in axonal development[2,38,39].

Huang *et al*., revealed that DG's structure might be a viable structural model for the development of novel candidates for the treatment of AD. Among these DG based structural derivatives, benzyl triazole derivatives showed promising neuroprotection against oxidative stress[40]. After 21 days of DG administration in Tri-Methyltin (TMT)-injected transgenic mice model 2576 showed a massive enhancement in Neuronal Growth Factor (NGF) along with a reduction in a stained plagues suggesting that DG might promote NGF synthesis in AD induced brain insults[41]. DG was also found to heal axonal atrophy and synaptic degeneration, as well as improve cognitive impairment in transgenic mouse and normal mouse models of AD. A study reviewed by Tohda *et al*., involving 28 healthy human participants (ages 20-81) in Toyama Prefecture, Japan, for a randomized control, double-blind, cross-sectional trial was conducted to rule out DG's low oral bioavailability and solubility. The test samples (placebo and DG) were formulated as soft capsules with olive oil, after 12 week of consumption significant improvement in the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) score. This study evidenced that the DG had a significant improvement in the cognitive functions in the test subjects[42]. Zhou *et al*., reported that the addition of indole fragments to DG showed enhanced neuroprotective effects, and the Electron Donating (ED) groups on indole ring were more beneficial than Electron Withdrawing (EW) groups and

thereby derivative of carbamate substituted by methoxy group, which exhibited excellent pharmacological actions against oxidative cellular damage caused by $H_2O_2$, 6-OHDA, and β-amyloid accumulation[43]. Askarani *et al.,* developed and produced a new series of DG derivatives, AChE/BuChE inhibitors were discovered and tested. The most important multiple biological activities including $A\beta_{1-42}$ aggregation, were used to further assessment of the active chemical. It was also found that $A\beta_{25-35}$ induced $PC_{12}$ cell damage was inhibited, with metal chelating characteristics and neuroprotective effect. They revealed that those compounds had a high level of potency, selectivity, and inhibitory efficacy against BuChE[44].

## 6.3 Cardiovascular Activity

Zheng *et al*., synthesized a DG derivative (acetylsalicylic acid diosgenin) that has reportedly prolonged the clotting time. It was suggested that the anti-thrombotic activity was stronger and considerably reduced the degree of thrombosis formation and had fewer adverse effects[45]. In a study conducted by Fu X L *et al*., DG was observed to be reacting with acetylsalicylic acid, ferulic acid, etc., In order to study their activity, the study results revealed that 3-disalicyl diosgeninogen and ferulic acid were the most active components. The anti-thrombotic activity of 3-acetylsalicylferulic acid ester acyl diosgeninogen was found higher and asserted that both could greatly reduce thrombosis and showed higher activity noticeably than the parent DG[46]. In multiple investigations dioscin and DG have been found to cause surge in the antioxidant enzymes such as Superoxide Dismutase (SOD) Glutathione (GSH) and Glutathione Peroxidase (GPx) level, while lowering the levels of oxidative products like Malondialdehyde (MDA), ROS in order to counteract oxidative stress. Both can inhibit the apoptotic genes Bax and Bax2. Caspase-3 and Bcl-2, an anti-apoptotic gene, are increased, whereas Bcl-2, an anti-apoptotic gene is downregulated inflammatory factors such as Tumor necrosis factor-alpha TNF-α, Interleukin -6 IL-6 and NF-κB are also reduced. Dioscin and DG may influence the nuclear factor erythroid 2-related factor 2 (Nrf2)/sirtuin 2 (Sirt2) signaling pathway and promotes antioxidant genes (HMOX1, KEAP1, NQO1 and GCLM) expression by inhibiting the Akt signaling pathway eventually lower the Doxorubicin (DOX) cardiotoxicity and inhibits endothelial Nitric Oxide Synthase (eNOS) in oxidative stress, as a result, NO levels are reduced, and arrhythmia is prevented. Furthermore, Dioscin suppresses hypertrophic cardiomyopathy by inhibiting PI3K/Akt/mTOR. It may also modulate the Transforming growth factor beta-1 (TGF1)/Smad3/JNK signaling pathways. Hence, its alleviating hypertrophic cardiomyopathy. Dioscin, has the ability to slow down lipid metabolism and has a therapeutic effect on total cholesterol, triglycerides, diabetic cardiomyopathy and myocardial I/R damage are also affected[47].

## 6.4 Diabetes Mellitus

DG lowered intestinal disaccharidases and α-glucosidase activity, which lowered the generation of hydrolyzed glucose and reduced carbohydrate absorption at the source[48]. Further, in Streptozotocin (STZ) induced diabetic rat model, DG was observed to prevent $Na^+$ binding in the intestine and lowered $K^+$ ATPase activity, which lead to decline in the glucose transport into the intestinal epithelial cells[49]. Further investigations revealed that DG suppresses the activity of Sodium glucose transport proteins-1 (SGLT-1) enzyme and glucose absorption mediated by SGLT-1[17] and improved insulin-dependent glucose absorption in 3T3-L1 cells by elevating the expression of Glucose Transporter type-4 (GLUT4) and its mRNA[50]. Also, DG led to a surge in serum and muscular Dehydroepiandrosterone sulfate (DHEA) levels, decreased blood glucose levels and enhanced the level of GLUT-4 translocation as well as Akt and PKC phosphorylation affecting glucose uptake and utilization in skeletal muscle thereby attenuating the symptoms and complications[51]. DG can control glucose metabolism and ameliorate insulin resistance through the Endoplasmic R (ER) signaling route and the PI3K/Akt signaling pathway, a direct result of its estrogen-like effect[50] and a signaling route that controls glucose metabolism. Further, thyroid hormone may be affected by DG and it increases insulin sensitivity and promotes metabolic clearance rate of insulin highly[52]. DG was found to stimulate the revitalization of pancreatic β-cells of STZ-induced diabetic rats, resultantly considerable increase in the amount of insulin produced thereby regulates blood glucose levels and plasma insulin levels[53]. Further, the elevated expression of CHOP, caspase-12, and caspase-3 in T2DM rats' pancreatic cells revealed that ER plays vital role in stress-induced β-cell damage. Treatment with DG resulted in considerable improvements such that, the protein levels were reduced, and protective effects were seen in β-cells via ER stress/UPR signaling control[54]. It has been reported that DG's hypoglycemic impact was the

cause of anti-hyperlipidemic effect and administration of DG improved insulin secretion, resulting in a substantial rise in plasma insulin levels and blood glucose, lipid levels and 3-Hydrpxy-3-Methylglutaryl-CoA Reductase (HMGR) levels were reduced considerably[55].

### 6.5 Anti-Cancer Activity

DG inactivates TNF-induced NF-κB activation and STAT-3 signaling pathways activated by IL-6 in tumor cells. It can thereby inhibit invasion, proliferation, and angiogenesis as well as apoptosis which is a key feature in cancer therapy[56]. DG notably suppressed the production of Azoxymethane (AOM) induced colonic aberrant crypt foci during the promotional stage of a rat colorectal tumor model[57]. Malisetty *et al.*, found that diosgenin reduced the occurrence and invasion of colon adenocarcinoma in an AOM-induced rat model by 60% and multiplicity of colon tumor (adenocarcinomas/rat) by 68% when a dose of 15 mg/kg was administered[58]. However, DG, when administered at dosages of 20, 100, and 200 mg/kg body weight in the diet did not elevate adenocarcinoma bulk in AOM/dextran sodium sulfate-induced colon aberrant crypt foci mouse model. Though, all three doses have shown a significant reduction in tumor multiplicity[59]. In another study where DG was administered intra-tumorally reduced the MCF-7 and MDA-MB-231 human breast cancer xenografts growth in mice[60]. Studies conducted by Yan LL *et al.*, found to decrease the formation of LA795 lung adenocarcinoma tumors in mouse by 33.94% utilizing inbred T739 strain[61]. DG was also found to suppress the formation of oral tumors at an oral gavage dose of 80 mg/kg in a Depot-Medroxyprogesterone Acetate (DMBA) induced hamster buccal pouch model[62]. In mouse xenograft model DG conjunction with thymoquinone inhibited tumor growth significantly[63]. Thus, DG inhibits tumor growth in preclinical cancer models via modulating several targets and two novel steroidal oxime compounds utilizing DG as the parent molecule in a recent study was synthesized by the researchers that exhibited strong antiproliferative effect on malignant cervical cells and human lymphocytes which further caused apoptosis and activation of caspase-3[64].

The semi-synthetic analogues of DG were found to have anti-proliferative effect against breast (HBL-100), lung (A549) and colon (HCT-116 and HT-19), malignant cells in a study by Mohammad *et al.* The powerful anti-proliferative action was mostly attributable to analogues with electron-withdrawing o-substituted R-moieties or the simple phenyl R-moiety connected to the parent DG, according to a structure-activity relationship research[65]. It was employed as a parent molecule to produce 1-hydroxysolasodine in another work[66] and it demonstrated considerable anti-cancer properties against Hepatocellular Carcinoma (HepG2), Prostate Cancer (PC3), and Cervical Cancer (HeLa), cells. When tested against various malignant cell lines, twelve distinct DG derivatives including a long chain fatty acid/ester of diosgenin-7-ketoxime showed activity against cancer. The anti-proliferative action of compound 16 in this series was linked to the reduction of LPS-induced TNF- and IL-6 activation in DU145 prostate cancer cells. The drug was also shown to have a maximum tolerated dose of 300 mg/kg in Swiss albino mice[67]. Ghosh *et al.*, in a recent study reported that the production of diosgenin functionalized nanoparticles of iron oxide having activity against breast carcinoma by inhibiting proliferation and migration and causing apoptosis[68].

## 7. Structure Activity Relationship (SAR) Anti-inflammatory

It is evidenced that n-(phenyl) methyl substitution in the 27$^{th}$ position of DG increases the anti-inflammatory activity in SH-SY5Y cell- line. Additionally, this substitution declines Nitric Oxide (NO) and Amyloidβ (Aβ) and ROS production. Computational docking model has also revealed that this substitution may have strong binding affinity towards the $A\beta_{42}$, iNOS and proinflammatory cytokines. Hence, n-(phenyl) methyl substitution in DG has strong anti-inflammatory, antioxidant and anti-Aβ activity[69]. Ibuprofen (2-(4-isobutylphenyl)-propionic acid) combined with DG derivatives employing 6-aminohexanoic acid at the 27th position has substantially proven for its anti-inflammatory activity in *in-vitro* and *in-vivo* inflammatory models[70].

### 7.1 Anti-Fungal Activity

Novel synthetic variants of Diosgenin's 3-O-tethered triazolyl have been discovered to show significant antifungal activity when electron withdrawing group such as -$NO_2$ and -CN substituted at the R-moiety of triazol ring especially against the *Candida albicans* and *Aspergillus fumigatus*[65]. In Addition, this substitution in DG improved anti-fungal activity against *Candida* type of fungi (Gram +ve), N-acyl, N-alkyl and N-dialkyl analogues

of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside exhibited potent antifungal activity[71].

### 7.2 Neuroprotection

The substitution of L-Ile (Isoleucine) aminoacid at 27th position have been showing promising angiogenesis and neuroprotective action in SH-SY5Y cell line models[72]. Similarly, Aβ and proinflammatory cytokines levels are reduced in the n-(phenyl) methyl substitution of Diosgenin derivatives[69].

### 7.3 Anti-Cancer Activity

Substitution of L-Tryptophan at 27th position in DG has shown potent cytotoxic activity, which was associated with Bcl2 associated -X (Bax) protein upregulation and B-cell lymphoma-2 (Bcl2) protein downregulation in K562 cell line[73] and similar pharmacological actions have seen in 1,3,4 thiadiazole substitution at 27th position[74]. Several amino acid derivatives such as (25*R*)-5α-spirostan-3β-yl L-serinate hydrochloride, (25*R*)-5α-spirostan-3β-yl L-glutamate hydrochloride, (25*R*)-spirost-5-en-3β-yl (*E*)-3-(3,4-dihydroxyphenyl) acrylate at 27th position exhibited more potent anti-tumor and immunomodulatory effect in *in vitro* studies[75]. Necrotic cell death was observed when treated with Dihydrodiosgenin derivatives in isolated pancreatic cells[76].

## 8. Toxicological Profile

DG is biocompatible and have low toxicity, a single dose of ethanol extracts of Dioscorea sp. (112.5-9000 mg/kg) consisting 28.34% of DG (31.7-2550.6 mg/kg) administered to rats in one short-term study have shown no signs of acute toxicity at 127.5, 255 and 510 mg/kg/day dosages, SD rats were orally given DG for 30 days in a sub-chronic toxicity study and that has proven no significant alterations in biochemical or hematological parameters. The main metabolite present in serum was DG[77,78]. A toxicological study utilizing root extract of *D. villosa* (DV) revealed that both short-term (5 g/kg, single dosage) and sub-chronic (1 g/kg/day, 30 days) treatments of rats yielded very minor alterations in hematological, biochemical, and histopathological markers[79]. Wojcikowski *et al.*, found no acute toxicity of kidney or liver when a DG crude extract produced from *D. villosa* was given orally at a level of 0.79 g/kg/day. In the same study, mice that were given a continuous therapy for 28 days developed renal fibrosis and liver inflammation. In a 90-day sub-chronic trial, mice were given fenugreek seeds containing 1%-10% DG showed no harmful signs[80]. However, an *in-vitro* investigation revealed that DG has harmful consequences that are mediated through genetic instability, decline in cell viability and a rise in the micronucleus frequency at doses >30 M was observed with DG treatment in HepG2 cell line and it had a substantial cytostatic effect on DNA damage[81].

## 9. Conclusion

Diosgenin is a type of steroid saponin found in plants that has various therapeutic potentials like anticancer, antiviral, antifungal, anti-inflammatory, and immunostimulatory properties. However, because of its pharmacokinetic properties like low bioavailability, its use is limited in the day-to-day practice of various systems of medicine. Many studies are being conducted to investigate and to improve the drug's therapeutic efficacy by modifying its pharmacokinetic properties. Hence, the current review focused on recent advances in DG and its derivatives in regards to its therapeutic approaches, as well as their physiochemical, pharmacological, and toxicological profiles. The drug is claimed to be efficacious at the optimum concentration, according to *in vitro* and *in vivo* studies and that the toxicity is rather minimal. Therefore, nano formulations are highly recommended for future investigations that will allow the therapeutic candidate to be developed successfully for treating the above-mentioned diseases.

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