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Review Article

Action of Diosgenin and Diosgenin-Containing Plants on Health and Female Reproduction -

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ABSTRACT

This review describes provenance, properties, general health effects, as well as the currently available knowledge concerning the action of diosgenin, diosgenin-containing plants and some of its metabolites on female reproductive processes. The analysis of the current literature shows that diosgenin-containing plants, diosgenin and its metabolites can via numerous signaling pathways affect a wide array of non-reproductive and reproductive processes. Diosgenin can affect proliferation, apoptosis and release of hormones and growth factors by healthy and cancer ovarian cells and uterine contraction. Diosgenin action on ovarian follicullogenesis, oogenesis and fecundity, as well as its applicability for promotion of female reproductive processes and treatment of their disorders are possible, but they require confirmation by animal and clinical studies. The possible areas of application of diosgenin for control of animal and human female reproductive processes and their disorders, as well as the missing knowledge in this area are outlined.

Keywords: Diosgenin; Health; Ovary; Proliferation; Apoptosis; Hormone; Fecundity

INTRODUCTION

In the recent decades, phototherapy has faced its comeback and increased popularity. One of the promising plant molecules, which attract attention of scientists, clinical doctors and the common public, is diosgenin. Diosgenin is considered as an important biological active constituent of medicinal and functional food plants. Its medicinal (preventive and curative) effects have been described in some reviews [1-3]. Nevertheless, the influence and applicability of diosgenin and diosgenin-containing plants for control of female reproduction have not been summarized yet. The present paper reviews provenance, properties, general health effects, as well as the currently available knowledge concerning the action of diosgenin, diosgenin-containing plants and some of its metabolites on female reproductive processes.

The present paper aimed to make a snapshot of the available information concerning production, metabolism, general physiological and therapeutic effects of apigenin. The main purpose of this review, however, is to describe, summarize and analyze the current knowledge concerning diosgenin effects on female reproductive processes and their dysfunctions, which have not been reviewed yet, but which could be useful for better understanding and application of diosgenin in reproductive biology and medicine.

Search for literature was performed in MedLine/Pubmed, Web of Science and SCOPUS databases between the year 2000 and 2021. In cases of repeated or conflicting information or references, more recent sources have been preferred. Words used to search were diosgenin, health, metabolism, ovarian, uterus, oocyte, embryo, fertility, and mechanisms.

Provenance and Properties

Diosgenin is a naturally occurring plant steroidal saprogenic. It is contained in relatively high amounts in the plants of *Agavaceae, Dioscoreaceae, Liliaceae, Solanaceae, Scrophulariaceae, Amaryllidaceae, Leguminosae*, and *Rhamnaceae* species. Diosgenin in commercial amounts can be isolated from tubers of wild yam (*Dioscurea villosa* Linn), rhizomes of yam (*Dioscurea zingiberensis* C. H. Wright, *Dioscorea nipponica,* Makino), seeds of fenugreek (*Trigonella foenum-graecum* Linn) or rhizomes *of* Himalayan trillium *(Trillium govanianum* Wall. ex D. Don) and ginger (*Costus speciosus* Koen ex. Retz). Tubers of several *Dioscorea* species contain more than 1% of diosgenin. *Trillium govanianum* and *Costus speciosus* contain around 2.5% and more than 2.12% of diosgenin respectively [4]. Diosgenin is poorly dissolved and extracted from plant tissues by water or water-based media. However, it is highly soluble in both nonpolar (chloroform, dichloroethane, propanol, ethyl acetate, and propylacetate) and partially polar (acetone, methanol, and anhydrous ethanol) solvents [4].

Diosgenin is produced mainly by hydrolysis of steroidal saponins in the presence of a strong acid, base, or enzyme catalyst. In addition, the microbial transformation is a promising, specific, environmentally friendly and cheap method for the production of diosgenin, too [4]. Furthermore, diosgenin can be a precursor for other biological active phytoestrogens [2] and for commercial production of diosgenin-derived steroidal drugs [5].

Diosgenin has low aqueous solubility, poor bioavailability and pharmacokinetics, and rapid disappearance from organism in in vivo conditions. To avoid these weak points of natural diosgenin, several semisynthetic diosgenin derivatives with various covalent linkage and attached functional entities with higher solubility in water, bioavailability and physiological activity have been designed and synthesized [4].

PHYSIOLOGICAL ACTION

The pre-clinical and clinical studies demonstrated that diosgenin and its metabolites have anti-cancer, neuroprotective, anti-diabetic, cardioprotective, hypocholesterolemic, gastro- and hepato-protective, anti-oxidant, anti-inflammatory, anti-osteoporosis, anti-asthma, anti-arthritis and other positive properties (see [1-3] for review).

For example, diosgenin can induce cancer cell death in vitro and in vivo, reverse multi-drug resistance in cancer cells and sensitize cancer cells to standard chemotherapy [5,6].

Diosgenin and its derivatives can be therapeutic agents for multiple disorders of central nervous system. In particular, those related to therapeutic efficacy for Parkinson's disease, Alzheimer's disease, brain injury, neuroinflammation, ischemia and stroke. They can improve learning and memory [4].

Fenugreek [7], yam [8] and their constituent diosgenin [9,10] can be applicable for reduction in plasma insulin, glucose, cholesterol and lipoprotein levels and treatment of diabetes and its complications including diabetic nephropathy, diabetic liver disease, diabetic neuropathy, diabetic vascular disease, diabetic cardiomyopathy, diabetic reproductive dysfunction, and diabetic eye disease, although the results of controlled clinical trials were controversial. Diosgenin can have potential anti-obesity action, although this action requires clinical evaluation, too [11].

The preclinical in-vitro and animal studies have shown that diosgenin has great potential in the treatment of various cardiovascular diseases, especially in atherosclerosis including endothelial dysfunction, lipid profile, and macrophage foam cell

formation, thrombosis, and inflammation during the formation of atherosclerosis [12]. Can effectively improve hypertrophic cardiomyopathy, arrhythmia, myocardial I/R injury and cardio toxicity caused by doxorubicin [13].

There are reports indicating the ability of diosgenin and its steroidal metabolites to prevent osteoporosis [14] and leishmania [2].

In folk medicine diosgenin and diosgenin-containing plants are used as galactagogues (provide lactation aid), but the results of pre-clinical and few clinical studies were inconclusive [5,15].

Diosgenin can be proposed as a male fertility-promoting drug, but this hypothesis has not been verified by the appropriate clinical studies [16].

Clinical studies did not demonstrate substantial toxic or any other adverse effects of daidzein [3,4,14] and daidzein-containing fenugreek [15].

Some physiological effects of daidzein could be not due to daidzein itself, but its metabolites. For example, daidzein metabolite dehydroepiandrosterone possesses a number of physiological and therapeutical properties similar to that of daidzein: antidiabetic, anticancer, anti-allergic, anti-obesity, anti-ageing, anti-dementia, anti-osteoporosis, anti-autoimmune disorders and anti-cardiovascular disorders. Moreover, dehydroepiandrosterone in turn can be an indirect precursor to estrogen and testosterone and other steroid hormones with biological active effects similar to daidzein [17]. Another diosgenin metabolite, sarsasapogenin, had even higher anti-leishmanial acetyl anti-cholinesterase and anti-butyryl cholinesterase activity, than its precursor [2]. Some diosgenin metabolites produced by gut microbiota can improve sport performance and their muscle recovery [18].

As it was mentioned above, the clinical application of diosgenin is hindered by its low aqueous solubility, poor bioavailability and pharmacokinetics, and rapid biotransformation under physiological conditions [3,4]. To avoid this concern, several novel diosgenin analogs and nano-formulations have been synthesized with improved pharmacokinetic profile and efficacy against cancer [5,6], neurodegenerative [4] and cardiovascular diseases [12,13].

These reports demonstrate the influence of diosgenin-containing plants, diosgenin and its metabolites on a wide array of physiological processes and illnesses, which show their high medicinal potential.

MECHANISMS OF ACTION

Diosgenin and its metabolite can affect numerous physiological processes and illnesses via inhibition of enzymes acetyl cholinesterase, butyryl cholinesterase and tyrosinase [2]. The in-vitro and animal studies demonstrated that the ability of diosgenin to suppress cancer and tumor development, neurodegenerative and cardiovascular and other diseases are mediated by several common mechanisms of action [1,3-5] listed below.

They reduce oxidative stress and peroxidation of DNA, lipids and proteins. They suppress immune response activity of immunocompetent T-cells and inflammation by inhibition of inflammatory cytokines, enzymes, adhesion molecules, PI3K/AKT/mTOR and JAK/STAT, WNT-beta-catenin intracellular pathways and transcription factor NF-κB . They activate cell death pathways (including promotion of pro-apoptotic bax and caspases and decrease in anti-apoptotic bcl-2) and resulted cytoplasmic/mitochondrial apoptosis. Furthermore, diosgenin has a unique structural similarity to estrogen. It can promote cellular growth/differentiation through the Estrogen Receptor (ER) cascade, MAP kinase and transcription factor PPARγ [1,3-5]. In addition, diosgenin suppress cancer cell functions via inhibition of PI3K/Akt/mTOR, and CDK5/ATM pathways and promotion of p53- and reactive oxygen species-induced autophagy, apoptosis and DNA damage [6]. Furthermore, diosgenin can prevent tumor metastasis by modulating epithelial-mesenchymal transition and actin cytoskeleton to change cellular motility, suppressing degradation of matrix barrier, and inhibiting angiogenesis [1].

Similar mediators of action have been proposed also for diosgenin-containing plants fenugreek [9] and yam [8].

The anti-obesity action of diosgenin can be due to its ability to suppress appetite at the level of CNS, to inhibit intestinal absorption of lipids, synthesis of lipids, adipogenesis and adipose tissue inflammation, and promoting fecal excretion of bile acids and triglycerides [11]. There are indications that diosgenin can inhibit pancreatic lipase, disaccharidase enzyme, antagonistic to in vitro lipogenesis [11].

Diosgenin metabolites generated by gut bacteria can affect transcription factors involved in mitochondrial biogenesis, antioxidant systems, glucose and lipid homeostasis, and DNA repair [18].

These data listed above suggest the multiple mechanisms of daidzein action. Some common (anti-oxidant, anti-inflammatory, anti-proliferative, pro-apoptotic) mechanisms mediate daidzein action on several processes and illnesses, but some mediators (for example, regulating adipogenesis and cell migration) are specific for particular daidzein targets.

EFFECTS ON FEMALE REPRODUCTIVE PROCESSES

Effect on ovarian and reproductive state

In experiments of [19], the feeding of mice with fennel (*Foeniculum vulgare* Mill.) containing diosgenin increased the number of growing, but not of small ovarian follicles. On the other hand, it remains not clear whether this effect on ovarian follicullogenesis was due to daidzein or other fennel constituents. The dietary pure diosgenin did not affect the number of follicles at each stage of follicullogenesis in murine ovaries, although it reduced the number of atretic follicles [20].

It remains not clear, whether and how daidzein can affect female reproductive organs other than the ovary. *Dioscorea villosa* (Wild Yam) root extract containing a high (< or =3.5%) amount of diosgenin did not affect rat uterus growth [21]. An extract of wild ginger (*Costus speciosus,* Koen) rhizomes containing diosgenin promoted contraction of strips of rat uterine myometrium [22]. On the other hand, diosgenin itself inhibited these contractions [22]. This observation indicates a relaxing action of daidzein on uterine tension and, maybe, on the resulting reproductive processes (embryogenesis, parturation etc.). This indication requires further experimental validation, however.

Therefore, the available information can indicate, but not demonstrate, the influence of diosgenin and diosgenin-containing plants on ovary or uterus. This information can indicate the

stimulatory action of diosgenin on ovarian follicullogenesis [20] and the inhibitory action on uterine contraction [22], and, therefore, on animal fecundity and gravidity. On the other hand, the differences in diosgenin-containing plants and diosgenin action on the ovary [19,20] and uterus [21,22] suggest, that plant action on these reproductive organs could not be due to presence of diosgenin.

Effect on ovarian cell functions

Diosgenin was able to increase accumulation of both proliferation and apoptosis markers in cultured porcine ovarian granulosa cells suggesting its ability to promote ovarian cell turnover [22]. On the other hand, in cultured human ovarian cancer cells diosgenin-containing extract increased apoptosis and reduced viability [24-26].

These observations indicate the stimulatory action of diosgenin on healthy, but the inhibitory action of this molecule on cancer ovarian cells.

Effect on oocytes and embryos

In traditional oriental folk medicine, many women eat diosgenin-containing fennel [19] or yam [20] for improvement fertility. The efficiency of this approach could be confirmed by studies of [27], which reported the ability of multinutrient containing diosgenin to improve oocyte quality, fertilizability and pregnancy rate in women during *in vitro* fertilization program. Nevertheless, it remained unclear, whether these benefits were induced by diosgenin or another constituent of multinutrient. The dietary pure diosgenin did not affect oocyte maturation, their quality and fecundity in mice [20].

Therefore, the available data indicates the possible positive effect of diosgenin on oocytes, but this effect has not been directly demonstrated yet.

Effect on reproductive hormones

In in-vitro experiments, addition of diosgenin inhibited progesterone and promoted testosterone and estradiol release in cultured porcine ovarian granulosa cells and isolated follicles [21,27]. In cultured rabbit ovarian fragments, diosgenin stimulated the release of both progesterone and insulin-like growth factor I (IGF-I) [23]. On the other hand, absence of diosgenin action on rat uterus indicates that it does not possess estrogenic effect and that it does not influence uterine estrogen receptors [21].

In in-vivo experiments of [20], feeding diosgenin to mice increased the level of anti-Mullerian hormone – marker of ovarian reserve in their plasma. No changes in expression of oocyte growth factors NOBOX, GDF9 and BMP15 involved in the control of ovarian follicle development were found.

Daidzein-containing drug reduced expression and reception of vascular endothelial growth factor (VEGF, promoter of tumor vascularization) in cultured ovarian cancer cells [26,29].

Therefore, daidzein can influence (mostly promote) the release of hormones and growth factors – stimulators of ovarian functions [30]. On the other hand, it can suppress production and reception of VEGF, a physiological stimulator of both ovarian follicullogenesis and ovarian tumor growth [24,26].

MECHANISMS OF ACTION ON FEMALE REPRODUCTIVE PROCESSES

The in-vitro experiments mentioned above demonstrated that diosgenin can affect ovarian and uterine cells directly, but not (or not only) through central (hypothamalo-pituitary) regulatory mechanisms.

The similarity of daidzein action on porcine whole ovarian follicles and isolated follicular granulosa cells [23] suggests that just granulosa cells are targets of diosgenin action in the ovary.

The action of daidzein on ovarian hormones and growth factors listed above suggests that these molecules could be extracellular mediators of diosgenin action on female reproductive processes. [23,24,26,28].

The action of daidzein on accumulation of markers and promoters of proliferation (PCNA) and apoptosis (bax) in cultured healthy porcine ovarian granulosa cells [23] suggests that these molecules could be intracellular mediators of daidzein action on healthy ovarian cells. Studies of daidzein action on ovarian cancer cells indicated much more potential intracellular mediators of daidzein on the ovary. In-vitro studies performed on human cancer cell lines [24-26,29] demonstrated that the pro-apoptotic effect of diosgenin-containing drug on ovarian cancer cells was associated with reduction in expression of phosphoinositide 3-kinase, phosphorylated AKT and phosphorylated p38 mitogen-activated protein kinase, extracellular signal-related kinase, Src family kinase, focal adhesion kinase and IKKβ kinase, up-regulation of apoptosis promoters bax and caspases 3 and 9. This observation indicates, that pro-apoptotic and maybe anti-proliferative action of diosgenin on cancer cells could be mediated by signaling pathways related to these protein kinases. [25] showed also the involvement of transcription factor NF-kB, which can suppress tumor growth and angiogenesis via up regulation of pro-apoptotic protein bax and down-regulation of anti-apoptotic bcl-2 and angiogenic vascular endothelial growth factor.

Diosgenin possess anti-oxidative and estrogenic properties which determine its suppressive action on non-ovarian cancer [1,3-5]. It might be proposed that these properties could determine its action on healthy female reproductive system and on its dysfunctions (cancer, polycystic ovarian syndrome etc.) listed above. Nevertheless, such mechanisms of diosgenin action on female reproductive processes have not been reported yet.

The current literature suggests the existence of multiple intracellular signaling pathways regulating proliferation and apoptosis of ovarian cells and therefore the fate of ovarian follicle and whole reproductive system. On the other hand, the main information concerning intracellular mechanism of diosgenin action on the ovary has been obtained in in-vitro experiment on ovarian cancer cell lines, whilst the physiological intracellular mediators of daidzein action on healthy reproductive system remain to be elucidated yet.

It is not to be excluded that the effects of diosgenin on female reproductive processes could be not due to daidzein itself, but its metabolites. At least, in experiments of [20], the daidzein action on murine reproductive processes were similar to the effects of its metabolite dehydroepiandrosterone.

POSSIBLE APPLICATION IN REPRODUCTIVE BIOLOGY AND MEDICINE

The *in-vivo* studies listed above demonstrated the stimulatory action of daidzein-containing plants and daidzein on ovarian follicullogenesis, oogenesis and fecundity [20,27]. In vitro experiments revealed the ability of diosgenin to promote ovarian cell turnover and release of hormonal stimulators of ovarian functions [23-30]. These

observations indicate that diosgenin or diosgenin-containing plants could be a biostimulator of animal and human female reproduction. This hypothesis, however, requires validation by appropriate in vivo pre-clinical and clinical studies.

It might be hypothesized, that if positive influence of daidzein on oocyte maturation and quality [27] would be confirmed, it could be used for improvement of in vitro maturation and fertilization and embryotransfer in programmes in animal production and assisted reproduction.

The ability of dietary diosgenin to prevent some age-dependent exhaustion of rat ovarian reserve and resulted fecundity [20] suggests potential applicability of diosgenin to prevent reproductive aging, to prolong reproductive cycle and to mitigate symptoms of menopause. Such effect could be examined on aged women.

The numerous reports concerning suppressive action of daidzein on cancer cells [24-26,29] suggest its applicability for prevention and treatment of ovarian cancer. This action should be however validated by in vivo and clinical studies. Furthermore, daidzein can be promoter of ovarian follicullogenesis [20] and steroid genesis [23,30] and follicle-stimulating anti-Mullerian hormone [20]. It might be hypothesized, that daidzein could be promising for treatment of polycystic ovarian syndrome, which is characterized by inhibition of these processes [31,32].

Finally, the ability of diosgenin to prevent toxic effects of metal nanoparticles on cultured ovarian cells [28] indicates that this molecule could be applicable as a natural protector against adverse effects of some environmental contaminants. Its ability to mitigate and prevent adverse effects of other environmental contaminants should be, however, examined in in vitro and in vivo animal and clinical studies.

Diosgenin application for improvement of reproductive processes and prevention and treatment of reproductive disorders could face the problem of its low solubility, bioavailability and rapid degradation [4]. From this viewpoint, the search for more active and less degradable metabolites and analogues of daidzein could be promising for treatment not only of non-reproductive [5,12], but also of reproductive disorders.

Therefore, the available results of in vitro and animal studies indicate the potential applicability of diosgenin and its metabolites or analogues for improvement animal and human reproduction and fecundity, prevention, and treatment of some reproductive disorders. These indications however require strong validation by further pre-clinical and clinical studies.

CONCLUSIONS AND POSSIBLE DIRECTION OF FUTURE STUDIES

The analysis of the current literature shows that diosgenin-containing plants, diosgenin and its metabolites can via numerous signaling pathways affect a wide array of non-reproductive and reproductive processes. Diosgenin can affect proliferation, apoptosis and release of hormones and growth factors by healthy and cancer ovarian cells and uterine contraction. Diosgenin action on ovarian follicullogenesis, oogenesis and fecundity, as well as its applicability for promotion of female reproductive processes and treatment of their disorders are possible, but they require confirmation by animal and clinical studies. The possible targets and mechanisms of diosgenin action on female reproductive processes are summarized in figure 1.

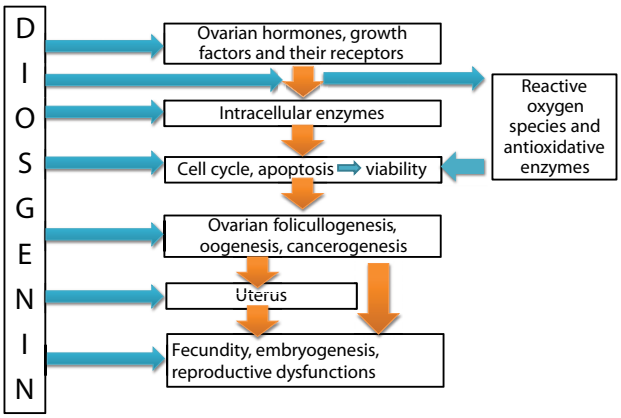

Figure 1: The possible targets and mediators of diosgenin action on female reproductive processes (simplified). Explanations are in the text.

Some queries, however, are to be addressed before application of diosgenin-containing drugs. The actions of diosgenin and diosgenin-containing plants are sometimes different, suggesting either the presence of plant biologically active molecules other than diosgenin or different forms or derivates of diosgenin in plant products. A number of mediators of diosgenin action on non-reproductive processes could be involved in mediating its effects on female reproduction, but they have not been detected yet. The causes of the opposite action of diosgenin on healthy (stimulatory) and cancer (induction of apoptosis and DNA damage) described above remain to be elucidated. The application of diosgenin is limited by missing or insufficient information concerning its in vivo action on animals and humans, as well as its low bioavailability. It is not to be excluded that replacement of diosgenin by its analogues or metabolites or by diosgenin-containing plant products could be more promising than application of diosgenin itself. Further profound studies are required to address these queries and to promote understanding and application of this promising molecule.

These studies could be focused on

- Understanding mechanisms of diosgenin and diosgenin-containing plants action
- Understanding causes of variability in diosgenin and diosgenin-containing plant effects
- Clinical studies of diosgenin and diosgenin-containing plant action on non-reproductive and reproductive disorders
- Design, testing and clinical application of diosgenin analogues with increased bioavailability and efficiency.

Such studies could expand the current knowledge concerning diosgenin and to define or to find the new approaches to its biomedical application.

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ETHICAL STATEMENT

The present study was performed according to international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights. The present publication reviews the data published previously, therefore the restrictions concerning animal experiments and clinical studies are not applicable here.

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