

Phytochemistry, Anthelmintic study of herbaceous aerial part of *Kallstroemia pubescens* (G. Don) and Review of Novel Effects of Diosgenin: A Plant Derived Steroid

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ABSTRACT

Diosgenin is an aglycone of the steroidal saponin, dioscin in yam and is a principal raw material for the industrial production of steroid drugs. It belongs to triterpene group and is of great interest to the pharmaceutical industry because of its estrogenic effect on the mammary gland. It plays an important role in cholesterol metabolism and it is responsible for morphological and biochemical changes in megakaryocyte cells. Diosgenin is an important steroidal metabolite used as a starting material for the synthesis of steroidal drugs, as it exhibits estrogenic activity. Work concluded that diosgenin possess therapeutic actions such as anti-inflammatory, anticancer. Its anti-inflammatory activity is mainly due to COX activity. Diosgenin is reported to stabilize lysosomal membrane and causes uncoupling of oxidative phosphorylation and having strong oxygen radical scavenging activity.

Keywords: Kallstroem; Diosgenin; Steroids; Sapogenins; Estrogenic activity.

Introduction

A spreading annual herb of waste places; introduced in habitat of waste places of Flora of Howrah. Plant is herbaceous, not woody and round. The large leaves are up to 8 inches long and are heart-shaped (cordate). The leaf blade's basal lobes are rounded. Leaf veins radiate from a single point. The leaves have long stems (petioles), and are alternate on the stem. flowers are small, yellowish and fragrant, hanging in relatively long clusters (panicles and spikes) up to 4 inches long. Plants produce "aerial part of the plant" that are attached closely to the stems where leaves attach to the stem (axil). They are grayish and somewhat irregular.

Compilation *Kallstroemia pubescens*

- *Kallstroemia pubescens*.
- *Kallstroemia maxima*.
- *Tribulus pubescens*.
- *Kallstroemia longipes*.
- *Tribulus maximus*.
- *Kallstroemia minor*.

Morphology

Flower: In axillary spikes, small. Flowering starts from September-November.

Fruit: Oblong.

Field tips: Stem twining to the left. Leaves are 7-13 nerved from base.

Leaf Arrangement: Opposite

Leaf Type: Simple

Leaf Shape: Ovate-suborbicular

Leaf Apex: Acuminate-shortly caudate

Leaf Base: Base is Cordate

Leaf Margin: leaves are Entire



Fig 1. Aerial part of *Kallstroemia pubescens*

General Habitat: Moist deciduous in the forests ie flora of Howrah, Indian distribution.

Diosgenin (25R-spirost-5-en-3 β -ol) is a hydrolysate of dioscin contained in the rootstock

of yam and it exists widely in the natural plant such as

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glucoside [1]. The discovery of diosgenin

in the tubers of the yield yam has made it one of the most researched and studied herbal product.

Many health benefits are associated with diosgenin, for example, prevention against cardiovascular disease, cancer and contraception [2, 3]. Diosgenin is an important steroidal metabolite used as a starting material for the synthesis of steroidal drugs, as it exhibits estrogenic activity [4]. Diosgenin has indicated effect of reducing the level of serum cholesterol [5, 6]. It is mainly used as the initial material for partial synthesis of oral contraceptives, sex hormones and other steroids. Diosgenin has received considerable attention because of the variety of them promising pharmaceutical properties [7, 8,9]. The consumption of diosgenin has positive actions on stress and inflammatory conditions.

Collection of Specimens

The different organs of this plant were cut and removed from the plant and fixed in FAA (Formalin 5ml + Acetic acid 5ml + 70 % Ethyl alcohol 90 ml) for histological studies, transverse sections of the different organs of the plant materials. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the schedule given by sass, 1940. Infiltration of the specimens was carried out by gradual addition of paraffin wax until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were section with the help of rotator microtome. The thicknesses of the section were 10-12 μ m. Dewaxing of the sections was performed by customary procedure (Johansen et al). The sections were stained with toluidine blue as according to the method prescribed by O'Brien et al. Wherever necessary, the sections were also stained with safranin and fast –green. The microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-chemical Standards

Physico-chemical constants such as consistency and organoleptic characters and the percentage of total ash, acid-insoluble ash, water-soluble ash and extractive values and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia (Anonymous, et al).

Phytochemical Screening of KP Extracted with Different Solvents

The extracts were tested for the presence of alkaloids, flavonoids, glycosides, phenols, resins, saponins, tannins, volatile oils, carbohydrates and amino acids using standard procedure.

Plant extraction

Five hundred grams of powdered of aerial parts of *Kallstroemia pubescens* were macerated separately in a 2 L flask using redistilled methanol as solvent for a period of 72 h with intermittent stirring with a glass rod and filtered using filter paper (Whatman No. 1, Whatman® Schleicher and Schuell). The combined filtrates were concentrated using Rotavapor (Rotavapor R-210; Buchi

Rotavapor) at a temperature of 40°C under reduced pressure. The extracts were stored at 4°C until needed for analysis.

Test organisms

Liver flukes (*Fasciola gigantica*, 2.2 to 4.4 cm in length) were obtained from freshly slaughtered cattle in the Bodija abattoir, in Ibadan metropolis (7.40°N, 3.92°E). Earthworms (*Pheritima posthuma*, 5.5 to 12.5 cm in length) were collected from the water logged areas of Chhatarpur, M.P. It was used due to its anatomical and physiological resemblance with parasitic gastrointestinal nematodes in human being).

Anthelmintic bioassay: The anthelmintic study of the flesh and peel extracts against the selected worms (*P. posthuma* and *F. gigantica*) was conducted according to the method described by Ajaiyeoba et al. with slight modifications. Plant extract (10 g) was dissolved in saline water to make stock solution and different concentrations of (100, 70, 50, 20 and 10 mg/ml) were prepared for the anthelmintic assay. Albendazole (10 mg/ml) was included as reference drug, while saline water was included as control. Standard drug and extract solutions were freshly prepared before starting the experiment. For the evaluation of each plant extract, five worms (same type) were placed in a 9 cm Petri dish containing 25 ml solution of methanol crude extracts of plant in the tested concentrations. The plant extract was dispensed into the Petri-dish before introducing the worms. Observations were made for the time taken until paralysis and death of an individual worm. Mean time for paralysis (P in min) was taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D in min) were recorded after ascertaining that worms neither moved when shaken rigorously nor when dipped in warm water (50°C). The LC50 was determined using a linear regression.

Epidemiologic Studies: Diosgenin has been used in traditional Chinese medicine for treatment of urethral and renal infections [10]. Diosgenin, made by hydrolysis of saponins, which were extracted from *Kallstroemia pubescens*, a plant which grows in India, shows presumed ability to minimize post-menopausal symptoms [11]. In Turkey, diosgenin is used as a good antispasmodic, that it can be used for cramps, coughs and for muscular spasms [12]. A new Indian source for diosgenin is *Costus speciosus*, which is used to induce apoptosis in cancer cells and to reduce high blood pressure [13]. Diosgenin extracted from *Trigonella foenum graecum* commonly called fenugreek, is a leguminous plant native to many Asian, Middle eastern & European countries, and is used as a hypoglycemic agent in type I and type II diabetes [14]. Over the past decade a series of preclinical & mechanistic studies have been conducted worldwide to understand the role of diosgenin as a chemopreventive agent against several cancers.

Experimental studies: Diosgenin plays an important role in the cholesterol metabolism Roman et.al, fractionated the liver using diosgenin to elevate biliary cholesterol and found that diosgenin can be absorbed through gut. A lot of experiments had been conducted to show that Diosgenin significantly induce apoptosis in various cell lines. Sahelian et al, studied the effects of sustained delivery of diosgenin on the adrenal gland of female rats

[15]. The changes in body weight, organ weight and histopathological changes in the adrenal gland of rats were observed and it shows that reduction in adrenal mass may pose a potential for major endocrine complications. Zhony Yao Za Zhi investigated the antitumor activity of diosgenin in vivo and invitro. Tumor growth inhibit rates were calculated. He showed that diosgenin has an obvious antitumor activity on S-180, Hep A, U14 transplant mice in vivo and L 929, Hela, MCF cells invitro [16].

Extraction of diosgenin: Methods of extraction of diosgenin from *Kallstroemia pubescens*, CH Wright include direct acid hydrolysis, spontaneous fermentation, supercritical CO₂ extraction and so on.

Recently, some researchers used a single enzyme such as cellulose, the amylase combined with

acid hydrolysis to treat drug material, demonstrating that about 70% diosgenin can be extracted from the material [17-20]. However, the activity of enzyme gradually reduces owing to the change of catalysis environment, so that the catalysis efficiency of enzyme will also reduce. Therefore, it is urgent to find efficient methods to enhance the stability of natural cellulase. Current methods that enhance the stability of natural cellulose include selection, protein engineering, enzyme immobilization, enzyme chemical modification and adding a co-solvent agent [21].

Production of Diosgenin: *Kallstroemia pubescens* is the dominant resource for the production of diosgenin. They were more than 1500t of diosgenin are produced annually. *Kallstroemia pubescens* cells aggregates were cultured in liquid modified MS medium supplemented with 2,4-D(2mg/l) and kinetine(0.1 mg/l). The cells were treated with different concentrations of ethylene-generating agent 2-chloroethylphosphonic acid (2-CEPA). 2-CEPA at concentrations of 50 mg/l, 100 mg/l elicited production of diosgenin [22, 23].

Diosgenin-Quantification: The diosgenin concentrations in a *Kallstroemia pubescens* tuber collection from EC, India were determined by HPLC and their percentages ranged from 0.02 to 2.64%. The average of diosgenin, recovery was 97%. Diosgenin was identified by gas chromatography-mass spectrometry (GC-MS) and coelution with authentic diosgenin standard in both HPLC and GCMS techniques. It shows that *Kallstroemia pubescens* is a potential new source of diosgenin [24].

Bioactive Compounds derived from Diosgenin: By utilizing the intact skeleton of diosgenin, OSW-1 and its analogues were synthesized. Its anticancer activities are 10-100 times more powerful than some of the well known anticancer agents currently in clinical use, such as mitomycin C, adriamycin and taxol [25]. From readily available diosgenin, 16 β -hydroxy-5 α -cholestane-3,6-dione, a metabolite from marine algae was synthesized. It is a potent oxysterol, which exhibit a number of biological activities, including inhibition of cellular proliferation and cytotoxicity associated with induction of apoptosis [26].

Certonardosterol D2, a polyhydroxysterol was stereoselectively synthesized from natural rich diosgenin, which possess a potent antitumor activity [27].

Regulation of Diosgenin Expression: Regulation of the diosgenin expression in *Kallstroemia pubescens* plants by different plant growth regulators was studied. Treatment with 10-5 and 10-4 M gibberelic acid led to 43% and 19% increases, respectively of diosgenin in 30-day-old whole plants. These increases might be associated with the action that this growth regulator has in stimulating plant growth and the biosynthetic pathway of this sapogenin. A similar increase was obtained with the 10-5M indole-3-acetic acid treatment. Treatment with 50 ppm ethephon increased the diosgenin levels observed in the leaves of 15 and 30-day-old plants, growth of the whole plant being substantially reduced at 30 days in comparison with the growth observed in control plants [28].

Dose: The amount of diosgenin to be administered per day is in the range 100 to 2000 mg, preferably 150 to 1200 mg, most preferably 300 to 1200 mg. This amount may be administered in a single dose or in more than one dose which may be taken at different times throughout the day.

Pharmacological properties of Diosgenin

Hypoglycemic activity: Consumption of commercial diosgenin demonstrated hypoglycemic properties, which are beneficial in diabetes by reducing intestinal disaccharides activities. It has been reported using experimental studies in diabetic male wistar rats, where there is a significant increase in lactase and maltase activities, reduced intestinal sucrose activity. The activity of glucose -6- phosphate was significantly increased [29].

Hypolipidemic and Antioxidant Activity: Oxidative stress has been suggested as a main risk factor in the development of atherosclerosis. Diosgenin enhanced the resistance to lymphocyte DNA damage caused by an oxidant challenge with H₂O₂. The hypolipidemic and antioxidative effect on rats fed with a high cholesterol diet supplemented with either 0.1% or 0.5% diosgenin for 6 weeks has been investigated. Diosgenin showed a decrease in the plasma and hepatic total cholesterol levels [30].

Neuroprotective Activity: Human Immuno deficiency virus (HIV) infection continues to rise in drug-abusing populations and causes a dementing illness in a subset of individuals. In-vitro studies showed that HIV proteins, gp120 and Tat, Tat + morphine caused increased neurotoxicity in human neuronal cultures with ApoE4 allele. A number of novel antioxidants has been screened and found that only L-deprenyl and diosgenin protected against the neurotoxicity of Tat + morphine [31].

Vasodilating Activity: Diosgenin is structurally "fairly similar" to progesterone. It is the precursor for the industrial large scale synthesis of different hormones including progesterone and norethisterone. The vasodilating effect of diosgenin was studied and it shows an acute, endothelium independent coronary artery relaxation [32].

Role in Cholesterol Metabolism: Diosgenin, structurally similar to cholesterol, has been shown to decrease cholesterol absorption and to increase biliary cholesterol secretion without altering either serum cholesterol or total biliary bile salt secretion [33]. It has been reported that increased biliary secretion of cholesterol and lipid

vesicles induced by diosgenin, has cytoprotective effects in the rat liver subjected to obstructive cholestasis [34].

Role in Melanogenesis: An increased level of melanin is characteristic of a large number of skin disease, including acquired hyperpigmentation conditions such as melasma, post inflammatory melanoderma and solar lentigo. Diosgenin inhibits the melanin content significantly [35]. Skin aging is a consequence of both programmed aging that occurs with time and aging caused by environmental factors such as exposure to ultraviolet rays. The supplementation of natural or synthetic diosgenin has anti-aging approaches [36].

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

Treatment of tumor cells with cytotoxic agents usually results in the breakdown of the cell cycle machinery, the cells subsequently entering into programmed cell death or apoptosis. Diosgenin plays a significant role in apoptosis. Diosgenin can inhibit proliferation via blocking cell cycle progression at the G2/M phase and subsequently progression to apoptosis in human leukemia K562 cells. Diosgenin can effectively inhibit the viability and proliferation of breast cancer cells MCF-7 [37]. Diosgenin induces differentiation of human erythroleukemia cell line (HEL TIB 180) through changing lipoxigenase activities. It also has been reported to induce apoptosis and cell cycle arrest in human osteosarcoma 1547 cell line. Diosgenin induced Hela cell apoptosis through caspase pathway [38]. Cyclooxygenase (COXs) are key enzymes in the conversion of arachidonic acid into prostanoids which are involved in apoptosis and inflammation. Two distinct COXs have been identified, COX-1 which is constitutively expressed and COX-2 which is induced by different products such as tumor promoter or growth factors. Diosgenin, induces apoptosis and its effects were tested on COX expression and activity in osteosarcoma cells [39].

Rheumatoid arthritis: RA is an inflammatory joint disease in which perpetuation of chronic synovitis leads to bone and cartilage degradation. Diosgenin causes an inhibition of the growth of fibroblast like synoviocytes from human rheumatoid arthritis, with apoptosis induction associated with cyclooxygenase-2 up-regulation [40]. Colon cancer is considered a preventable disease. However, there seems to be no decline in the incidence of colon cancer and many of the risk factors associated with colon cancer prevail. In-vitro experiments indicated that diosgenin inhibits cell growth and induces apoptosis in the HT-29 human colon cancer cell line in a dose dependent manner [41]. Fatty acid synthase (FAS) expression is markedly elevated in HER 2-overexpressing breast cancer cells. In this, diosgenin found to be effective in suppressing FAS expression in HER 2-overexpressing breast cancer cells and preferentially inhibited proliferation and induced apoptosis in HER 2-overexpressing cancer cells [42].

Adverse Effect: As an herbal extract, diosgenin appears to be free of any major adverse effects. In this, diosgenin found to be effective in suppressing FAS expression in HER 2-overexpressing breast cancer cells and preferentially inhibited proliferation and induced apoptosis in HER 2-overexpressing cancer cells.

Drug also shows sustained delivery of diosgenin on the adrenal gland of female rats [43]

Conclusion

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

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