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REVIEW ARTICLE

Wathania Coagulans Derivatives As Potent Inhibitors Against HMG COA Redutase

Fearoz Khan[1], Shameem A. Qureshi[2], Bilal Azmi[3], S.M Naeem[4], Muhammad Shoib[5]

[1]*Rahman Medical College Peshawar*
[2]*Department of Biochemistry, University of Karachi*
[3]*Department of Biochemistry, Dow University of Health Sciences, Karachi.*
[4]*Saidu Medical College, Swat*
[5]*Bannu Medical College, Bannu*

Correspondence: *S.M.Naeem*
E-mail: smnaeem2000@yahoo.com

Abstract

The medicinal property of whole plant of Withania coagulans, which belongs to Solanaceae family in Ayurvedic medicines has been widely studied and emphasized. Whole plant and its fractions as well as extracts have been used as therapeutic agents for various illnesses, including cardiovascular diseases, hyperglycemia and hyperlipidemia. It has also been used as hepatoprotective, free radical scavenger, anti-inflammatory, antibacterial as well as central nervous system depressant. Its cytotoxic, immunomodulating and antitumor properties have also been studied. This review article is aimed to highlight the pharmacological activities of W. Coagulans.

Keywords: Solanaceae, Withaniacoagulans, Withanolides.

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Introduction

Phytochemicals are vital in the development of novel options of treatment against various illnesses. Numerous therapeutical agents have been discovered in the pharmacological screening of compounds, extracted from plant sources. Many are traditionally been regarded as a rich source of lead, including quinine, cocaine, morphine, digitalis, and others of similar nature. Various societies have been traditionally using different herbs as part of their indigenous therapeutic protocol. In India, Withania coagulans, also known as Dunal (synonym: Puneeriacoagulans Stocks), Indian rennet, vegetable rennet (English), Indian cheese maker, Panirkephool, Panir band, Punirdodi (Hindi), and Ninggushuiqie (Chinese), is found widely occurring in drier parts of the subcontinent[1]. Western Asia, tropical areas and Indian Subcontinent regions are considered home to the plant. A survey of the literature has shown that in various traditional systems of medicine the plant has been recommended for the treatment of various disorders[2].

Taxonomical Classification

Kingdom: Plantae, Plants; subkingdom: Tracheobionta, Vascular plants; super division: Spermatophyta, Seeds plants; division: Angiosperma; class: Dicotyledons; order: secure Tubiflorae; family: Solanaceae; genus: Withania; species: coagulans[3].

Phytochemistry

W. coagulans, Dunal seeds contain free sugar 17.8% having, D-galactose and D-arabinose in the ratio 1: 1, with traces of maltose. Absence of a b-galactosidic linkage has been reported in enzymatic studies of the polysaccharide[4]. The seeds contain fatty oil to the extent of 12–14%. Fruits fraction of the plant contains a hydrocarbon and sterol dihydrostigmasterol which have been extracted from the unsaponifiable portion. High percentage of beta sitosterol and linoleic acid are reported in the oils obtained from the seeds of W. Coagulans. These are reported to have hypocholesterolemic effect[5]. W. coagulans is rich in steroidal lactones, which are known as withanolides

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(Figure 1). Withanolides are naturally occurring polyhydroxy C28 steroidal lactones. In the basicstructure of all withanolides a six- or five-membered lactone or lactol ring is attached to an intact or rearranged ergostane skeleton. They give a positive Dragendorff's test even though they are not N-containing. On spraying the TLC with H_2SO_4–MeOH they give a characteristic blue colour spot. This class of compounds has not been found in all members of the Solanaceae family. The occurrence of withanolides, however, is not restricted to Solanaceae. They have also been reported from marine organisms (soft corals) and from members of plant families Taccaceae and Leguminoseae[5].

Isolated Compounds

Plants, that produce withanolides are having a characteristic ability of introduction of oxygen atom at every position of carbocyclic skeletal and side chains of numerous compounds. Modifications either of the carbocyclic skeleton or of the side chains result in manynovel structural variants of withanolides. Previous phytochemical examination of the whole plant resulted in the isolation of 25 compounds, including 24 withanolides and one dimericlignan, bispicropodophyllinglucoside[6] . In total nine compounds have been isolated from the fruits of W. coagulans, including ergosta-5, 25-diene-3b, 24ξ-diol and sitosterol-b-D-glucoside along with withanolides. Five withanolides have been reported to have been isolated from the root of the plant (Table 1, Figure 2)[7]. Withanolides with regular17b-oriented and unusual 17a-oriented side chains have been reported from W. coagulans. Withaferin A (32) is a common withanolide present in many plants

(Withania, Acnistus etc.). Hairy roots, induced by the inoculation of leaf sections of W. coagulans with Agrobacterium tumefaciens strain C58C1 (pRiA4), have the capacity to produce withanolide A and withaferin A. Time course studies of withanolide production showed that withanolide A accumulated at the first part of the culture, whereas the maximum accumulation of withaferin A occurred near the end of the culture period[8].

Pharmacological Properties

Plant berries are used for coagulation of milk[3]. It occupies a prominent position in Unani, Ayurvedic, and ancient Indian traditional medicine. Various biological and pharmacological activities have been reported from compounds, isolated from the plant. The fruits have been reported to have antiemetic, sedative, alterative and diuretic effects. These are also useful in chronic liver problems. It has also been used as blood purifier. Similarly, its uses in curing in dyspepsia, flatulent colic and intestinal infections. These are also used as anti-asthmatic as well as for the treatment of biliousness and stranguary[7].

Hepatoprotective Activity

Fruits extract (aqueous) of the plant has shown exerting hepatoprotective action. Since the steroidal compounds (glucocorticoids) having anti-inflammatory properties are used in some hepatic disorders, 3-b-hydroxy-2, 3-dihydrowithanolide F has been screened for its hepatoprotective effect. It has shown hepatoprotective activity against CCl4-induced hepatotoxicity in adult albino rats of either sex (150–200 g) at 10 mg/kg (i.p.). The protective effect was assessed by observing pentobarbitone (30 mg/kg; i.p.) -induced hypnosis, the determination of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels, and histopathological examination of hepatic tissues after staining with haematoxylin and eosin solutions. Concomitant treatment of the rats with 10 mg/kg withanolide protected the liver significantly ($P < 0.05$)[9].

Anti-inflammatory Activity

Markedly anti-inflammatory activity of the alcoholic extract of the plant has been report in acute inflammation induced with egg albumin and subacute inflammation induced with formalin as well as granulation tissue formation through cotton pellet method[10]. 3-b-Hydroxy-2,3-dihydrowithanolide F has reportedly exhibited significant anti-inflammatory action at 10 mg/kg in subacute inflammation like granuloma formation as well as formalin-induced arthritis in rats. The effect was noted to be comparable with the anti inflammatory activity obtained with 50 mg/kg phenylbutazone and 10 mg/kg hydrocortisone. It, however, did not have any significant action in acute models of inflammation[11,12].

Antihyperglycemic Activity

Lowering of blood glucose and serum cholesterol has been noticed by administration of an aqueous extract of fruits of W. coagulans @ 1g/kg; p.o. Lowering of serum lipid peroxide (LPO) and hepatic LPO levels has also been reported in streptozocin-induced diabetic rats after seven days of treatment ($P < 0.001$)[13]. This lipid lowering activity in streptozocin-induced diabetic rats might have

been due to prevention of associated atherogenesis and other secondary complications of diabetes mellitus. Its serum LPO and liver LPO reducing activity is suggestive of the possible prevention of lipid peroxidation and hence protecting tissues from the adverse actions of free radicals. It has also found to have significantly ($P < 0.01$) decreased the blood glucose level in normal rats (at 1 g/kg; p.o.)[14]. Compounds isolated from the plant like Coagulin C, withanolide F, 17b- hydroxy withanolide K, coagulanolide ((17S, 20S, 22R)-14a, 15a, 17b, coagulin L, 20b-tetrahydroxy-1- oxowitha-2, 5, 24-trienolide) and coagulin L have been found to have for antihyperglycaemic activity in the normoglycaemic rats group (SLM) and in the streptozocin induced diabetic rat groups (STZ). These also showed improved glucose tolerance up to 29.8% in SLM and 23.3% in STZ-induced diabetic rats at a dose of 100 mg/kg body weight. Significant hypoglycemic effect of the isolated compounds was also studied and even a significant lowering of post prandial blood glucose level to the extent of 22.7 % was studied in mice at the dose of 50 mg/kg body weight for 10 consecutive days[15,16].

Hypolipidemic Activity

Reduction in blood cholesterol level by giving 1 g/kg; p.o. aqueous extract of fruits of W. coagulans in Triton induced hyperlipidemic rats, compared to the control untreated animals. High fat diet-induced hyperlipidemic rates has also shown significant reduction in body weight, blood cholesterol and total lipids, after administration of extract at the same dose for seven weeks showed a significantly reduced body weight. The animals treated with aqueous extract of fruits of W. coagulans and the reference drug Navakaguggulu showed less degenerative changes along with microvesicular fatty changes[17].

Free radical scavenging Activity

Scavenging of free radical activity in, in-vitro model, by the the aqueous extract (2 mg/ml) has been reported, using 1,1-diphenyl-2- picrylhydrazyl (DPPH). This method, used to determine free radical scavenging activity, was based on the reduction of a methanolic solution of the coloured free radical DPPH. The decrease in absorption of DPPH at its absorption maximum of 517 nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. The activity was expressed as the effective concentration at 50%. The presence of free radical scavenging potential might help in protecting against oxidative damage to pancreatic beta cells[18].

Antimicrobial Activity

Antimicrobial and antifungal properties have been reported in the withanolides isolated from the ethanolic extract of the whole plant and leaves, respectively[19,20]. Exhibition of antibacterial activity of withaferin A against Gram positive microorganisms at the concentrations 6–100 µg/ml, whereas it was inactive against Gram-negative bacteria or non filamentous fungi has been studied[21]. Minimal inhibitory concentrations (mg/ml) have been found for withaferin A: 6.25 for Staphylococcus pyogenes, 12.5 for Sarcinialutea, 100 for Streptococcus pyogenes, 200 for Streptococcus viridans, 25 for Bacillus subtilis, 50 for Corynebacterium diphtheriae, 6.26 for Bacillus anthracis, 200 for Escherichia coli, and 200 for Pseudomonas aeruginosa[21]. Antifungal activity against Aspergillus niger, Candida albicans and Taenia rubrum at doses of 12.5–50 µg/ml, 100, 150 and 200 µg/ml has been shown. It was found to be more inhibitory to the filamentous fungi than to the yeast group of fungi.[29] The volatile oil obtained from fruits of W. coagulans had antibacterial activity against Staphylococcus aureus and Vibrio cholerae and was also found to have anthelminti cactivity.[37] 17b-Hydroxywithanolide K (3) exhibited antifung activity against human pathogens Nigrospora oryzae, Aspergillusniger, Curvularia lunata, Stachybotrysatra, Allescheriaboydii, Drechslera rostrata, Microsporumcanisand Epidermophyton floccosum and plant pathogen Pleurotuso streatus (minimal inhibitory concentration 300 µg/ml)[22,23].

Cardiovascular Effects

An alcoholic solution of 3b-hydroxy-2, 3-dihydrowithanolide F at the dose of 5 mg/kg, exhibited a moderate fall of blood pressure (34 ± 2.1 mmHg) in mongrel dogs (weight 12–15 kg). The hypotensive response was blocked by atropine (2 mg/kg) butnot by mepyramine (2 mg/kg) and propranolol (1 mg/kg). At the same dose, the hypotensive response was less with a suspension of the withanolides. On administration of a 10 mg/kg bolus dose in alcohol, a depression of the S-T segment was caused in ECG studies of dog. A 2 mg dose in suspension produced a positive inotropic and chronotropic effect in perfused frog heart. The heart rate increased from 61.2 ± 1.39 to 77 ± 1.94 beats/min ($P < 0.01$). In rabbit Langendorff preparations 2 mg withanolide produced negative inotropic and chronotropic effects. The

heart rate decreased from 71 ± 2.4 to 19 ± 0.28 beats/min. In rat limb preparation, 1 mg withanolide caused insignificant (P > 0.05) vasoconstriction. On administration of 5 mg/kg it increased the rate and depth of respiration. The rate of respiration increased from 18 ± 1.4 to 65 ± 5.3 breaths/min in dogs, which was insignificant (P < 0.01)[24].

Central Nervous System Depressant Activity and Acute Toxicity

The total extract of W. coagulans fruit has been reported having central nervous system (CNS) depressant activity in mice, rabbits and dogs. The extract was hypotensive in animals and had respiratory stimulant and smooth muscle relaxant activity. Alcoholic extract, total alkaloids and aqueous extract at doses of 1 g/kg, 200–400 mg/kg and 5 mg/100 g exhibited CNS depression in albino rats characterized by sedation, reduced exploratory, spontaneous activity and hypothermia. At the same doses but administered 30 min before a hypnotic, they potentiated pentobarbitone sleeping time in rats. They did not show any analgesic and diuretic activity in albino rats. Alcoholic extract (1 g/kg) and total alkaloids (200–400 mg/kg) did not protect against convulsions induced by pentylenetetrazol (70 mg/kg). They increased the lethal effect of amphetamine in aggregated mice. [33] 3b-Hydroxy-2, 3-dihydrowithanolide F (26) was tested for its CNS depressant activity. It was found nonlethal to mice up to a dose of 625 mg/kg (i.p.). It had not showed analgesic, hypothermic or local anaesthetic activity[25,26].

Immunomodulating Activity

Withaferin A has been reported to have both immune activating and immunosuppressive properties, even at a low dose of 10 mg/kg for six consecutive days. Withaferin A was found also to impart immuno activation by specifically inducing proliferation of peritoneal macrophages in mice but not in splenocytes, resulting in regression of tumour cells in a mouse carcinoma model, which was persistent even after passive transfer of the serum or macrophages of the treated mice into another model[27].[Withaferin A has reported to have specific immunosuppressive effects on B and T lymphocytes in humans as well as on mice thymocytes. It also inhibits E rosettes and EAC rosette formation by normal human T and B lymphocytes at low concentrations. It was demonstrated to affect the functional activity of normal human T lymphocytes as assessed by a local xenogeneic graftversus host reaction. It had specific action on antigenrecognition as well as proliferative capacity of T lymphocytesand B lymphocytes[28]. 5,20a (R)-dihydroxy-6a,7a-epoxy-1-oxo-(5a)-witha-2,24-dienolide is known to show immunosuppressant activity inspleen cell culture. On administration of doses above 1 mg/ml, it has inhibited proliferation of murine spleen cell cultures. A solution of 5,20a(R)-dihydroxy-6a,7a-epoxy-1-oxo-(5a)-witha-2,24-dienolide in dimethyl sulfoxide was mixed with RPMI 1640 medium to achieve a fine suspension (0.37%dimethyl sulfoxide)[29]. Coagulin H has shown effects on the immuneresponse, e.g. an inhibitory effect on lymphocyte proliferation, and expression of interleukin-2 (IL-2) cytokine. A complete suppression of hytohaemagglutinin-activated T-cells was observed at ≥2.5 µg/ml coagulin H and this suppression activity was similar to that of prednisolone, acommonly used immune modulating drug. Coagulin H alsosignificantly inhibited IL-2 production by 80%. Dockingstudies predicted that coagulin H bound to the receptor binding site of IL-2 more effectively than prednisolone. Based on the computational and the experimental results,coagulin H was identified as a potential immunosuppressive candidate[30].

Antitumour Activity

Withaferin A demonstrated marked tumour-inhibitory activity in-vitro, against cells derived from human carcinoma of the nasopharynx (KB). Withaferin A inhibited RNA synthesis of Sarcoma-180 ascites tumour cells. At 40 µg/ml, within 30 min of incubation it has showed inhibition of RNA synthesis of more than 50%. It inhibited protein synthesis of Sarcoma-180 cells. In this way, withaferin A inhibited transcription and translation processes of these cells. At concentrations of 0.01–0.5% it showed inhibition on the growth of roots of Allium cepa by arresting the cell division at metaphase after 2-h treatment.[42] Withaferin A acted as a mitotic poison via arresting the division of cultured human larynx carcinoma cells at metaphase. It showed a similar, but less marked effect on HeLa and embryonal chicken fibroblast cells. Withaferin A inhibited human umbilical vein endothelial cell (HUVEC) sprouting in three-dimensional collagen-I matrix at doses which were relevant to nuclear factor-kappa B-inhibitory activity. Withaferin A inhibited cell proliferation in HUVECs at doses that were significantly lower than those required for tumour cell lines through a process associated with the inhibition of cyclin D1 expression[31].

Cytotoxic Activity

In-vitro effects of Withaferin A on P388 cells have been studied. The cytotoxicity was calculated from the utilization of precursors in protein and nucleic acid synthesis and from capacity to suppress cell proliferation. Withaferin A stopped cell proliferation and, at the same time, killed the cells. Cytotoxicity was found to be due to a double bond at position C2–3; on dissociating this bond the cytotoxicity markedly decreased. A dissociation of the double bond at C24–25 or a removal of OH group from C27 did not cause any significant changes in the biological effects. An addition of a carbonyl group at C4 increased the effect. As withaferin A promptly reacted with L-cysteine, it was presumed that one of the possible target sites in the cell might be the SH groups of enzymes, which react with the lactone and epoxide groups of the agent[32].

Conclusions

W. somnifera and W. coagulans. W. somnifera, closely resembling species are known by the name 'Ashwagandhain' in Hindi and 'Indian ginseng' in English, having withanolides as the principal compounds found in both. Withaferin A is a major compound found in W. somnifera, whereas, coagulin L has been found in major amounts in W. coagulans. Where antihyperglycaemic leads from W. coagulans have been identified, it is still to be determined in W. somnifera. A unique thio-dimer of withanolide named Ashwagandhanolide has been found in W. somnifera. W. somnifera has an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory activities. Various compounts present in the plant display antibacterial, antihyperglycaemic, hypolipidaemic and antitumoral actions. W. coagulans had the greater therapeutic value overall. The variety of activities reported in the extracts, fractions and withanolides isolated from W. coagulans provide promising evidence for future research. Withanolides could achieve an important place in the world of modern drugs. Isolation on a large scale, chemical transformations and synthesis of the active compounds will definitely enhance their pharmacological value. The pharmacophores of various pharmacologically active withanolides have not yet been identified. Clinical trials using the active compounds for a variety of conditions need conducting. All these advantages prove the significance of W. coagulans in natural product research.

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