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ANTIDIABETIC ACTIVITY OF FRUITS OF WITHANIA COAGULANUS DUNAL IN STREPTOZOCIN INDUCES DIABETIC: A REVIEW

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ABSTRACT:

Herbal medicine is a drug or preparation made from plants or parts of plant (leaves, root, bark, seeds and flowers) valued for its medicinal, aromatic or savoury qualities to treat the symptoms of wide range of problems from depression to cold and flu. Insulin in humans composed of two α- subunit and two β- subunit linked by disulfide bond to constitute the β-α-α-β heteropetramer. Literature review of Withania coagulans prove that this plant have medicinal use and also have antidiabetic activity. The aim of work is "Evaluation of antidiabetic activity of fruits of *Withania coagulans dunal* in alloxan induce diabetic rat". Administration of an aqueous extract of fruits of *W. coagulans* (1 g/kg; p.o.) significantly lowered the blood sugar, serum cholesterol and serum lipid peroxide (LPO) and hepatic LPO levels in streptozocin-induced diabetic rats after seven days of treatment. In the present study, plant was collected from AMAZONE online site, and Fruits of plant were collected from the natural habitats. The plant & fruits were identified & authenticated from BN college , udaipur.A voucher specimen of the plant was deposit in the laboratory.

INTRODUCTION

1.1 Herbal medicines

Herbal medicine is a drug or preparation made from plants or parts of plant (leaves, root, bark, seeds and flowers) valued for its medicinal, aromatic or savoury qualities to treat the symptoms of wide range of problems from depression to cold and flu.[1]

1.2 Poly herbal formulations

Polyherbal formulations are defined as combination of two or more active drugs in single dosage form. Polyherbal formulation formulated in such a way that it should have advantages over single formulation product in therapeutic effect, safety or compliance.

1.3 Diabetes

Diabetes mellitus is metabolic disorder caused due to disturbed glucose metabolism and in these blood sugar level increases because of deficiency in the production of insulin by the pancreas. It is characterized by polydipsia, polyphagia, hyperglycemia, polyurea and due to disturbance in carbohydrate, protein and lipidmetabolism associated with absolute or relative deficiency in insulin secretion or insulin action.[5]

1.4 World Diabetes Day(WDD)

WDD introduced in 1991 by the International Diabetes Federation (IDF) and Word Health Organization (WHO). In December United Nation recognized diabetes as a global threat and designated 14th November as the World Diabetes Day.[10]

1.5 Historical background

In 1675, Thomas willis added the world mellitus, from latin meaning "honey" a reference to the sweet taste of the urine. In 1776 Matthew Dobson confirmed that the sweet taste was because of an excess of a kind of sugar in the urine and blood of the people with diabetes.[11]

1.6 The Pancreas

Pancreas is a flattened elongated organ laying against the posterior wall of the upper abdomen.[13] The pancreas has both endocrine and exocrine function. The exocrine secretions are mostly the digestive enzymes such as pancreatic amylase (a carbohydrate digestive enzyme), trypsin and chymotrypsin (protein digestive enzymes) and pancreatic lipase (a triglyceride digestive enzyme).

1. Alpha cells (α-cell): which constituent about 17% of islet mass and secretglucagon that raises blood sugar level.

2. Beta cells (β-cell): which constituent about 70% of islet mass and secret insulin that lower blood sugar level.

3. Delta cells (D-cell): which constituent about 7% of islet mass and secret growth hormone release inhibiting hormone or somatostatin. In pancreas it inhibits the secretion of glucagon and insulin. In hypothalamus it inhibits the synthesis and release of growth hormone while in GIT it inhibits release of gastrin.

4. The F cells: which constituents the remainder of the islets mass, secret pancreatic polypeptide which regulate the release of pancreatic digestive enzymes and concentration of gall bladder.[15]

1.6.1 The role of pancreas in the human body

The pancreas plays a primary role in the metabolism of glucose by secreting the hormone insulin and glucagon. The islets of langerhans secrete insulin and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose level.[16]

1.7 Insulin

Insulin, the hypoglycemic-anti-diabetic factor polypeptide is a polypeptide hormone secreted by the β cells of islets of langerhens of pancreas. Insulin was the first hormone to be isolated from animal source in pure enough form to be administered therapeutically and it was the first mammalian peptide hormone whose biosynthesis by recombinant DNA-technology was achieved. Insulin is present to facilitate the uptake of glucose, it penetrates most tissue slowly.

1.7.1 Chemistry, biosynthesis of insulin

Prepro insulin is a bio-precursor molecule of insulin. In this two polypeptide chains A and B which are connected to each other by C peptide chain. The proteolytic degradation of proinsulin, in golgi apparatus by proteases, result in the formation of insulin along with C-peptide in equimolar concentrations, which are then stored in the pancreatic β-cells granules.

1.7.3 Pharmacological action of insulin and Mechanism of action[19]

Insulin is a major anabolic hormone. It promotes the uptake the storage of glucose, fats and proteins through effect on liver, muscle and adipose tissues. It is also known as storage hormone. It influences the cell growth and metabolic function of various tissues. Excess secretion of insulin leads to hypoglycemia while lake of insulin release leads to hyperglycemia.Rapid action of insulin include carbohydrate, protein and fat metabolism –

- **Carbohydrate metabolism:**

(a) In liver cell - It decrease glycogenolysis (inhibit conversion of glycogen to glucose) by inhibiting glycogen phosphorylase and increase glycogenesis (promot hepatic glucose storage as glycogen) by activating glycogen synthesis.Insulin also decrease gluconeogenesis and inhibit conversion of noncarbohydrate substrate to glucose.

(b) In Muscles – Promote glycogenesis by translocation of intracellular glucose transportar and increase glycolysis (conversion of glucose and ADP into lactate and ATP)

(c) In Adipose Tissue – it facilitate glucose uptake. It increase intracellular glucose oxidative metabolism. Glycerol is estrified with fatty acids to form triglyceride.

- **Protein Metabolism:**

(a) In Liver cell – It decrease protein breakdown and inhibits oxidation of amino acids to ketoacids.

(b) In Muscles – it increase protein synthesis and increase amino acid uptake by muscle cells to produce a net positive nitrogen balance.

- **Fat metabolism:**

(a)In liver cell – it increases lipogenesis (conversion of glucose & other neutirients to fatty acids)

(b) In Adipose tissue – it increase fatty acid synthesis and triglyceride formation and storage, decrease lipolysis and blunt lipolytic action of adrenaline, growth hormone and glucagone. Long term effect of insulin regulates gene transcription and stimulates cell proliferation and differentiation. It regulates protein synthesis and growth regulation.

Mechanism of action of insulin:-

Insulin in humans composed of two α- subunit and two β-subunit linked by disulfide bond to constitute the β-α-α-β heteropetramer.β subunit contain tyrosine kinas residue when insulin bind to α – subunit at the outside of cell surface the tyrosine kinas activity in β subunits is stimulated.

1.8 Types of Diabetes:

(1) Type-1 diabetes / Insulin-dependent diabetes mellitus (IDDM)

In type-1 diabetes there is complete absence of insulin due to destruction of the β-cells in the islets of Langerhans. Destruction of β – cell is due to genetic, poor diet and environmental factors and infection with Coxsackie virus B or Encephalo-Myocarditis virus can also cause destruction of β cell of pancreas.

(2) Type-2 diabetes / Non-insulin-dependent diabetes mellitus (NIDDM) In type 2 diabetes body does not produce enough insulin, and if produce then this insulin is less effective. Type 2 diabetes is due to defects in the islet beta cells, so that less glucose is produced, and to an impairment of insulin's ability to stimulate the uptake of glucose in muscles and other tissues. The cause of this insulin resistance has not yet been fully established, but may involve defects in the action of insulin after it has bound to the insulin receptor on the surface of cells.[21]

(3) Type-3 diabetes / Prediabetes

It is also known as “impaired glucose tolerance,” is a health condition with no symptom. It is almost present before a person develops the more serious type-2 diabetes. More than 50 million people in the U.S. over age 20 have prediabetes with blood sugar that are high than normal, but are not enough to classified as diabetes. Early diagnosis and treatment of prediabetes may prevent type 2 diabetes.[22]

(4) Type-4 diabetes mellitus

It is also called “Gestational Diabetes Mellitus”. This type of diabetes observed in the 4-5% pregnancy and usually during the postpartum period, no genetic predisposition. The most plausible cause is that during pregnancy, the placental hormone promotes insulin resistance.[1] women who develop type-1 diabetes during pregnancy and women with undiagnosed symptom of type-2 diabetes mellitus that is discovered as gestational diabetes.

Causes

- **Heredity** - people who belong to family background having history of diabetes are 25% more prone to develop diabetes.
- **Diet** – it is one of the major factors for diabetes. Eating too much carbohydrate, protein, fat is all harmful to body.

Obesity – excess body weight as compare to the height of individual serve as predisposing factor for diabetes.

Virus infection – certain virus like coxsackie B virus may infect pancreas, leading to destruction of β-cells of islets.

Age – particularly above 40 years of age, in them to develop the chances of diabetes.

Emotional stress – high stressed life, busy.

People who smoke – frequently are highly susceptible diabetes.

High alcohol uptake.

High blood pressure.

High triglyceride level.

1.10 symptoms of diabetes 25

Increase thirst, increase hunger, dry mouth, nausea and occasionally vomiting, frequent urination, unexplained weight loss, fatigue, blurred vision, labored breathing and frequent infection of skin, urinary tract and vagina.

1.11 complications of diabetes 26

- **Diabetes kitoacidosis** – With out insulin, the cell starving of the energy. The body will break down fat cells. Products of this fat breakdown include acidic chemicals called ketones build up in the blood, causing an increase in acidity. The liver continues to release sugar it store to help out. Since the body cannot use this sugar without insulin, more sugar piles into the blood. The combination of high excess sugars, dehydration, and acidic build up is known as "ketoacidosis".

- **Diabetic Retinopathy** – This eye problem occur in 75% - 90% adults, having diabetes for more than 15 years. Diabetic retinopathy in Type-1 diabetes is rare before puberty no matter how long they have had the disease. Medicalconditions such as good control of sugars, management of high blood pressure, and regulation of blood fats are important to prevent retinopathy.

- **Diabetic neuropathy** - About 35% - 45% of people with type 1 diabetes develop kidney damage, a condition called neuropathy. This complication carries risk of serious illness such as kidney failure and heart attack.

- **Poor blood circulation** – damage to nerve and hardness of the arteries decreased sensation and poor blood circulation in the feet. This can lead to increase risk of injury. Damage to nerve may also lead to digestive problem such as nausea, vomiting and diarrhea.

- **Dehydration** - The increase of sugar in blood can cause an increase in urination. When kidney loses the glucose through the urine, a large amount of water is also lost, causing dehydration.

- **Diabetic Coma** - when a person with type 2 diabetes become severely dehydrated and is not able to drink enough fluid to make up for the fluid losses, they may develop this life threatening complication.

1.12 Diagnosis of diabetes[27,28,29]

1. Urine test: Urine is tested for reducing sugars like glucose, galactose, sucrose etc. and is also tested for ketone bodies.

2. Fasting blood glucose level: fasting blood glucose in morning normally 50-90 mg/dl and 110 mg/dl is considered to be the upper limit of normal.The principle involves conversion of blood sugar to gluconic and hydrogen peroxide by glucose peroxidase. Blood collection in sodium fluoride bulb retains the sugar value for several hours at room temperature and several days if kept in refrigeration with 10% loss. Arterial blood sugar level is 20 – 50 gm higher than venous blood sugar level in normal adults. In diabetes arterial and venous difference is lost, meaning glucose is not utilized by tissue.

3. Acetone breath: Small quantity of aceto-acetic acid is present in blood which increases greatly in diabetes mellitus which is volatile and vaporized into the expired air. Diagnosis can be done simply by smelling the breath of the patient.

4. Insulin assay: plasma insulin can be measured by radio immune assay or enzyme immune assay.

5. Oral glucose tolerance test: when a normal fasting person is orally given with one gram glucose per kg, the blood sugar level rises from about 90 mg/dl often above 140 mg/dl.On digestion of glucose the blood glucose level rises abnormally and glucose level falls back to normal value only after 4-6 hrs or sometime it may fail to fall down in normal value.

6. C- peptide assay:C – peptide measurement is more sensitive than insulin assay because its level is not affected by insulin therapy. Patients may be maintained on insulin therapy while accessing their β-cell functions.

7. Glycated hemoglobin HbA_{1c} test:A major advance in the laboratory monitoring of diabetic patient has been the introduction of measurement of glucose modified hemoglobin. Normally HbA_{1c} account for 4-6% of the total hemoglobin.

1.13 Management of Diabetes Mellitus

Diet, exercise, modern drugs including insulin and oral administration of hypoglycemic drugs such as sulfonylurea and biguanides manage the pathogenesis of diabetes mellitus. Insulin plays a key role in glucose homeostasis along the side of a counter regulatory hormone glucagone, which raises serum glucose. Carrier proteins are essential for glucose uptake into cells. In individuals with type 2 diabetes, a common sequence of therapy starts with diet treatment and exercise followed by oral Antihyperglycemic agents.

Summary of streptozotocin-induced diabetes

Streptozotocin (STZ) is one of the most prominent cytotoxic glucose analogue used in diabetic research. STZ is an antimicrobial agent with diabetogenic properties and also used as an alkylating agent in chemotheray. The effects of STZ on insulin and blood glucose concentrations reflect its toxic induced abnormalities in beta cell functions. The low affinity of GLUT2 glucose transport in the plasma membrane causes STZ to be selectively accumulated in the pancreatic beta cells

resulting in inhibition of insulin secretion (Schein et al., 1967; Rakieten et al., 1963; Bergevin, et al., 1974). In the present study, streptozotocin (STZ) was used to induce diabetes in rat

Chemical Name- 2-Deoxy-2-([(methylnitrosoamino)carbonyl]amino)-D-glucopyranose

Chemical structure - It has a cytotoxic methylnitrosourea moiety (Nmethyl-N-nitrosourea) attached to the glucose (2 deoxyglucose) molecule; glucosamine derivative (see figure below)

Chemical properties- Hydrophilic, beta cell-toxic glucose analogue. It is relatively stable at pH 7.4 and 37OC (at least for up to 1 h)

Chemical reactivities -DNA alkylating agent and protein alkylating agent Toxicity mode DNA alkylation.

AIMS AND OBJECTIVES

Literature review of Withania coagulans prove that this plant have medicinal use and also have antidiabetic activity. The aim of work is "Evaluation of antidiabetic activity of fruits of *Withania coagulans dunal* in alloxan induce diabetic rat".

Objectives of study

Work performed in III semester

1.Collection, authentication, storage and size reduction of plant material.

2. Standardization of plant material according WHO.

3. Extraction of dried fruits of Withania coagulans Dunal.

4. Phytochemical investigation of extract by chemical test.

Work performed in IV semester

1. Extraction of plant material using Soxhlet apparatus.

2. Biochemical analysis**:**

In biochemical analysis following parameter will evaluated

- Glucose level
- Hb_1Ac (Glycosylated hemoglobin)
- SGPT (Serum Glutamic Pyruvic Transaminase)
- SGOT (Serum Glutamic Oxaloacetic Transaminase)
- Creatinine

rt used	

1.14.2 Introduction of *Withania coagulans dunal*

Withania coagulans dunal is very well known for its ethno pharmacological activities. The *W. coagulans*, is common in Iran, Pakistan, Afghanistan and East India, also used in folk medicine.[36]*Withania* is a genus of flowering plant in the family Solanaceae with 23 species that are part of North Africa, the Middle East and Mediterranean region and extend to South Asia. *Withania coagulans* is an important medicinal plant. In view of its, varied therapeutic potential, it has also been the subject of numerous pharmacological investigation. There are two species of Withania one is *W. somnifera*and *W. coagulans*. *W. coagulans*has received attention in the recent years due to its ethno medicinal properties.[37]

1.14.3 Withania coagulans: A remedial drug

Withania coagulans is used in chronic complaints of liver. A composite Ayurvedic herbal hepatoprotective medicine 'Liv-52' contains extracts of Withania coagulans and W. somnifera. They are also used in dyspepsia, flatulent colic and other intestinal infections. In some parts of Pak-Indian sub-continent, the berries are used as a blood purifier. The twigs are chewed for cleaning of teeth and the smoke of the plant is inhaled for relief in toothache.

1.14.4 Taxonomical classification[40,41]

- Kingdom: Plantae Plants.
- Subkingdom: Tracheobionta, Vascular plants.
- Super division: Spermatophyte, Seeds plants.
- Division: Angiosperma.
- Class: Dicotyledons.
- Order: Tubiflorae.
- Family: Solanaceae.
- Genus: Withania.
- Species: coagulans.

1.14.5 Distribution[42]

Drier parts of Punjab, Gujarat, Simla and Kumaon in India, Baluchestan in Iran,Pakistan and Afghanistan.

1.14.6 Synonyms[42]

- English: Vegetable Rennet.
- Indian-Indian Cheese-maker, panir ke phool, Punir dodi.
- Unani- Desi Asgandh.
- Siddha/Tamil-Ammukkura.
- Chinese- Ning gu shui qie.

1.14.7 Vernacular Name of Withania Coagulans[43]

The plant is known by different names in different local languages such as;

- Bengal - Asvagandha
- Bombay - Kaknaj
- Gwalior - Asgandha
- Panjab - Khamjaria, Khamjira, Panir
- Sindhi - Punirjafota, Punirband
- Persian - Kaknajehindi, Punirbad
- Arabic - Javzulmizaja, Kaknajehindi
- Canares - Asvagandhi
- Telgu - Panneru-gadda

- Urdu - Hab kaknaj

1.14.8 Botanical Description[44,45]

- Shrub - Rigid, gray-whitish small shrub.
- Length - 60-120 cm tall.
- Leaves - 2.5-7.5 cm long and 1.5 cm broad, lanceolate oblong, sometimeovate, obtuse and narrow at the base.
- Flowers - 7-12 mm across, yellowish, dioecious and polygamous in nature.
- Flowering period - January to April.
- Berries are about 7-12 mm in diameter, red, smooth.
- Seeds - 2.5–3 mm in diameter, dark brown and ear shaped, glabrous withsharp fruity smell.

1.14.9 Part used[44,45]

Whole plant, roots, leaves, stem, green berries, fruits, seeds and bark are used.

- Fruits: Carminative, depurative, used for dyspepsia, flatulence and strange.
- Berries contain a milk co-agulating enzyme, esterase, free amino acids, fatty oil,an essential oil and alkaloids.
- The milk coagulating activity is due to the presence of an enzyme, underoptimum conditions.
- Seeds: anti-inflammatory, emetic, diuretic, emmena-gogue.
- Leaf: alterative, febrifuge.

1.14.10 Phytochemistry[44,45,46]

Aqueous and methanolic extract from the W.coagulans show different chemical constituents such as alkaloids, steroids, tannins, saponins, carbohydrate, protein, amino acid & organic acid.

- Seed contain 17.8% free sugar, D-glactose & D-arabinose, maltose, fatty oil.
- Fruits contain milk-coagulating enzyme, two esterases, free amino acids, fatty oil an essential oil and alkaloids, hydrocarbon triacontaine, sterol dihydrostigmasterol. Amino acid like proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine asparagine, cysteine and glutamic acid. Alkaloidal fractions have been isolated from the alcoholic extract of the fruits.
- Leaves contain four steroidal lactones called Withanolides, Withaferin-A, 5, 20α(R)-dihydroxy-6α,7α-epoxy-1-oxo-(5α)-with a-2,24-dienolide and two minor withanolide, of which one is probably 5α, 17α-dihydroxy-1-oxo-6, 7α-epoxy-22R-witha-2,24-dienolide.

Basic structure of W.coagulans

W. coagulans has a steroidal lactones known as withanolide. This is a group of steroidal lactones found among members of Solanaceae.Withanolides are naturally occurring polyhydroxy C28 steroidal lactones. In the basic structure of all withanolide 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 H2SO4–MeOH they give a characteristic blue color spot. This class of compounds does not occur in all members of the Solanaceae family. However, the occurrence of withanolide is not restricted to Solanaceae.

Basically there are two major groups of withanolide as follows:

(A) Withanolides with an unmodified skeleton

- With a regular β -oriented side chain
- With an unusual α -oriented side chain.

(B) Withanolides with modified carbocyclic skeletons or side chains.

These withanolide are initially classified on the basis of the chemo types of the Withania species depending on the region of the collected plant. Chemically, these compounds may be classified as ergostane derivatives from their structural pattern; these can be broadly divided into seven groups.

(1).5β, 6 β -epoxides

(2) 6 α, 7 α -epoxides

(3) 5-enes

(4) Intermediate compounds

(5) 5 α, 6 α -epoxides

(6) 6 β, 7 β –epoxides

(7) Phenolic withanolide.

Among these, the 5β, and 6β -epoxides are most common. Most of the compounds possess a 4β -hydroxy1 group.

1.14.12 Pharmacological properties of *Withania coagulans*[48,49,50]

The berries of the plant are used for milk coagulation. A number of reports have proven that the Withanolides isolated from *W. coagulans* possess interesting biological activities. The fruits of the plant are sweet and have been reported to have sedative, emetic, alterative and diuretic effects. They are useful in chronic complaints of the liver.

➢ **Antihyperglycemic activity**

Administration of an aqueous extract of fruits of *W. coagulans* (1 g/kg; p.o.) significantly lowered the blood sugar, serum cholesterol and serum lipid peroxide (LPO) and hepatic LPO levels in streptozocin-induced diabetic rats after seven days of treatment. Such lipid lowering activity in streptozocin-induced diabetic rats may have helped in preventing associated atherogenesis and other secondary complications of diabetes mellitus.

➢ **Hepatoprotective activity**

The aqueous extract of fruits of this plant has been shown hepatoprotective activity. 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 haematoxylinn and eosin solutions. Concomitant treatment of the rats with 10 mg/kg Withanolide protected the liver significantly.

➢ **Cardiovascular effects**

An alcoholic solution of 3b-hydroxy-2, 3-dihydrowithanolide F (26) 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) but not 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 ($P < 0.01$).

➢ **Nervous system depressant activity and acute toxicity**

The total extract of *W. coagulans* fruit has been reported to have central nervous system (CNS) depressant activity in mice, rabbits and dogs. The extract washypotensive 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.

➢ **Immunomodulating activity**

Withaferin A (32) has been reported in various studies to possess both immunoactivating and immunosuppressive properties, even at a low dose of 10 mg/kg for six consecutive days. Withaferin A was found also to impart immunoactivating by specifically inducing proliferation of peritoneal macrophages in mice but not in splenocytes, resulting in regression of tumor 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. Withaferin A had specific immunosuppressive effects on human B and T lymphocytes as well as on mice thymocytes. It inhibited E rosettes and EAC rosette formation by normal human T and B lymphocytes at very low concentrations. It was demonstrated to affect the functional activity of normal human T lymphocytes as assessed by a local xenogeneic graft versus host reaction. It had specific action on antigen recognition as well as proliferative capacity of T lymphocytes and B lymphocytes.

EXPERIMENT RESULT AND DISCUSSION

In the present study, plant was collected from AMAZONE online site, and Fruits of plant were collected from the natural habitats. The plant & fruits were identified & authenticated from BN college , udaipur.A voucher specimen of the plant was deposit in the laboratory.

The authenticated part was subjected to physiochemical characterization. The dried fruits of the plant were used to prepare extraction. The extract was filtered, distilled off and concentrated on water bath and finally reduce to dryness. The concentrated extracts were stored carefully for physiochemical investigation. Then all extracts were subjected to detailed phytochemical investigation.

Result and discussion: The moisture content is found, when I was dried the powder drug. Significance of moisture content determination is related to stability of powdered drug because excess of moisture content cause breakdown of important constituents by enzymatic activity and may encouraged the growth of yeast and fungi during storage.

. Extraction procedure

- **Alcoholic extract**

Dried fruits of *W. coagulans Dunal* were used for the alcoholic extract. Fruits were collected, calyx and pedicle were removed. Fruits were soaked in distilled water and were kept for 7 days. After 7 days extract was filtered and used for phytochemical investigation.

5.3.3 Qualitative chemical investigation of extract

Qualitative chemical tests were conducted for the fruits of *Withania coagulans Dunal* to identify the various phytoconstituents. The various test and reagents used are given below:

1.) Test for alkaloids

- **Dragendorff's Test**: To 1 ml of the extract, added 1 ml Dragendorff's reagent, and orange red precipitate indicated the presence of alkaloids.
- **Wagner's Test**: To 1 ml of the extract, added 2 ml of Wagner's reagent. Theformation of a reddish brown precipitate indicated the presence of alkaloids.
- **Mayer's Test**: To 1 ml of the extract, added 2 ml of Mayer's reagent, a dull white precipitate revealed the presence of alkaloids.
- **Hager's Test**: To 1 ml of the extract, added 3 ml of Hager's reagent, the formation of yellow precipitate confirmed the presence of alkaloids.

2.) Tests for carbohydrate

- **Molish test:**Aqueous or alcoholic solution of substans + 10% Alc. Solution of ANapthol shake + con.c sulphuric acid along the side of test tube. Violet ring atthe junction of two liquids. Show the presence of carbohydrate.
- **Fehling's test**:- 2 ml of fehlings solution A + 2 ml ofB + 2ml of sugar solution(66.7 gm glucose + 100 ml water) + boil. Yellow or brick red ppt show theprescence of reducing sugar.

Benedict's test:- 5 ml of benedicts' reagent + 3ml of sugar solution + boil for2 min and cool. Green yellow or red ppt shows the prescence of reducing sugar.

Barfoed's test:- 2ml of test solution + 2ml of barfoed reagent + boil on waterbath. Brick red ppt at the bottom of test tube indicate the presence ofMonosaccharide.

Seliwanofl's test:- 3ml of seliwanofl's reagent + 1ml of sugar solution + boilfor 2min. If red ppt is observed then it show the presence of ketose likefructose and sucrose.

3.) Test for saponin

Foam test: 2 ml of aqueous extract and 2 ml of alcoholic extract were taken in a test tube and shake with little quantity of water. Foam produced persists for ten minutes it indicated the presence of saponins in both extracts while no foam produced in water extract indicate the absence of saponins.

Froth Test: 2 ml of aqueous extract and 2 ml of alcoholic extract were taken in two test tubes. Diluted with 20 ml of distill water and was shaken in a measuring cylinder for 15 minutes. No formation of thin layer of foam indicates the absence of saponins in aqueous and alcoholic extract. While the formation of thin layer indicate the presence of saponins in both extracts.

) Test for glycoside

Borntrager's Test: 2 ml of aqueous extract of water and alcoholwere taken in different test tubes. Extract hydrolyzed with hydrochloric acid and treated with chloroform. Chloroform layer was separate out. To the separate layer of chloroform few drops of ammonia solution was added and formation of red color show the presence of glycoside.

Baljet test: Alcohol and water extracts were taken and add few drops of sodium picrate solution. Absence of orange colour shows the absence of glycosides.

Legal test: Alcohol and water extracts were taken in different test tubes. Add 1 ml of pyridine solution and few drops of sodium nitroprusside. Formation of pink to red color shows the presence of glycosides.

Kellar killiani test: Alcohol and water extract were taken in different test tubes. Both the extract hydrolyzed with hydrochloric acid Add glacial acetic acid, few drops of 5% w/v solution of ferric chloride and conc. Sulphuric acid along the side of test tubes. No color formation shows the absence of glycosides.

5.) Test for Flavonoids

Ferric Chloride Test: The extract was dissolved in 3 ml of ethanol. 1 ml of solution was taken in a test tube and few drops of neutral ferric chloride solution was added, purple colour appeared in the test tube indicated presence of flavonoids.

Lead Acetate Test: 1 ml of extract was taken in a test tube and few drops of lead acetate solution was added. Yellow colored precipitate appear in the test tube indicates presence of flavonoids. Ether, chloroform and alcoholic extracts were found to contain flavonoids.

Thin layer chromatography analysis

TLC was conducted on silica gel G coated plate with a view to ascertain the number of phytochemical constituents present in the different extracts. Chromatographic studies were performed in following manner:

- **Preparation of TLC plate**

Glass plates of size 15×4 cm were used. Slurry of silica gel G was prepared in distills water. The slurry was poured on to the plates to get uniform layer of about 0.25 mm thickness. The plates were first air dried for 10 to 20 minutes. These plates were then activated by heating at 105°c for 60 minutes in oven.

- **Preparation of mobile phase and TLC chamber**

The TLC chamber made of glass was used for thin layer chromatographic studies. The TLC chamber was lined on three of the inner walls with filter paper. About 100 ml of each solvent system mentioned as table no 16 were introduced into TLC chamber. After 10 to 15 minutes, the chamber becomes saturated with solvent vapors.

- **Application of sample**

To mark the origin, a line was drawn 2 cm from the bottom of the activated plate, with the help of a pencil. Sample of different extract were prepared and samples were applied point wise on the drawn line on activated silica gel G coated plates using thin capillary tubes.

- **Development of TLC plate**

After application of sample, TLC plate were introduced into saturated TLC chamber in such a way that solvent did not slop over the starting line and washed the substance away. TLC chamber was kept in covered condition. When the solvent travelled up to an optimum height, plate was removed from chamber. Time required in optimum travelling of solvent varied from 15-20 minutes. Distance traveled by the solvent system was noted.

- **Detection of spot**

Spot were detected using iodine chamber, UV chamber and detecting reagent.

- **Determination of R_f value**

The chromatographic measurement of the substance in thin layer chromatography was done with the help of R_f value which were calculated by using following formula:-

$$Rf\ Value = \frac{\textbf{Distance travel by the spot from the origin}}{\textbf{Distance travel by the solvent from the origin}}$$

SUMMARY

Withania coagulans dunal is an ethno-botanical plant, belongs to the family Solanaceae.*Withania* is a genus of flowering plant

in the family Solanaceae with 23 species that are part of North Africa, the Middle East and Mediterranean region and extend to South Asia. *Withania coagulans* is an important medicinal plant. In view of its, varied therapeutic potential, it has also been the subject of numerous pharmacological investigation.

Conclusion

In the present study, the dried fruits of *Withania coagulans dunal* were subjected to successive extraction by using water & alcohol. Both the extracts were subjected to phytochemical investigation and revealed presence of glycosides, saponins, phytosterols, flavonoids, tannins, phenolic compounds and proteins.

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