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PHYTOCHEMICAL EVALUATION & PHARMACOLOGICAL SCREENING OF ANTIDIABETIC ACTIVITY USING ETHANOLIC EXTRACTS OF PULICARIA DYSENTERICA & BROSIMUM ALICASTRUM ON EXPERIMENTAL ANIMALS

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Abstract:
This project is focused on the study of anti-diabetic effects of the leaf extract of Pulicaria dysentrica &brosimum alicastrum on streptozotocin induced male Wister rats. To evaluate the anti-diabetic activity of ethanolic extract of pulicaria dysentrica & brosimum alicastrum, this is tested by phytochemical analysis, GCMS analysis, In vivo & Invitro screening models. Oral dosing of plants extracts in different doses alleviates rats blood serum levels. This may be achieved by estimating the BGL, SGOT, SGPT, TGR, TCL, ALP, HDL, VLDL, LDL and the histopathological study of the pancreas. The rats' pancreas was dissected out from one animal of each group for histopathology examination for evaluation of hyperplasia, islets damage, and restoration of size of organ. The pancreas microscopy of toxic, standard &toxic group is compared. It was found that with the plant extract the hyperplasia is repairing when compared to toxic but is less compared to standard control. The study asserts that further research needs to be undertaken on the benefits of pulicaria dysentrica and brosimum alicastrum in diabetes. Keywords: anti-diabetes, ethanol, streptozotocin, pulicaria dysentrica, brosimum alicastrum, hyperplasia.

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INTRODUCTION:

1.1 HERBAL MEDICINE: The ability or way of using herbs and herbal preparations for good health and to avoid, improve or to heal the disease A plant used in herbal medicine. The phrase "herb" has been derived from the Latin phrase, "natural" and an vintage French phrase "herbs". Now a days, herb refers to any a part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, similarly to a non-woody plant. Earlier, the time period "herb" was best applied to non-woody plants, which encompass those who come from trees and shrubs. These medicinal plant lives are also used as meals, flavonoid, remedy or perfume and additionally in positive non secular sports. The term "medicinal plant" encompasses diverse forms of flora used in herbalism ("herb logy" or "natural remedy"). It is the use of plants for medicinal functions.

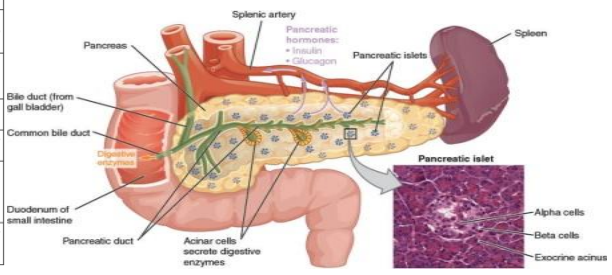
1.2 DEFINATION OF DISEASE: Diabetes mellitus is the major endocrine disorder affecting nearly 2.5%of the population all over the world. It is deadly disease that affects estimated 300 million people worldwide. In the indigenous Indian system of medicine, good number of plants were mentioned for the cure of diabetes. Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels, that result from defects in insulin secretion, or action, or both. commonly referred to as diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas1.3 TYPES Type I Diabetes:- It is also referred as IDDM (Insulin dependent diabetes mellitus or Juvenile diabetes).This results when the pancreas produces insufficient amounts of insulin to meet the body's needs. Approximately 10% of the diabetic population is composed of Type I or insulin-dependent diabetes. Type II Diabetes:-It is also referred as NIDDM (Non insulin-dependent diabetes mellitus or "adult-onset" diabetes). This result when the pancreas produces insulin, but the cells are unable to use it efficiently; this effect is called 'insulin resistance'. About 90 to 95 percent of people with diabetes have type 2. 1.4 CAUSES Insufficient production of insulin, Inability of cells to use insulin, Heredity, Increasing age, Obesity, Impaired glucose tolerance . PATHOPHYSIOLOGY: Type 1 (IDDM):In this type beta cell destruction in pancreatic islet is occurs due to presence of autoimmune (type 1a) antibody in blood which results in decrease in circulating insulin level. Type 2 (NIDDM): There is no loss or moderate reduction in beta cell mass. Abnormality in gluco-receptor of beta cell reduced sensitivity of peripheral tissues to insulin Excess of hyperglycemic hormones (glucagon)/ obesity.

SYMPTOMS: Increased urine output, Weight loss despite an increase in appetite., Patients also complain of fatigue, nausea and vomiting, Sweet taste in the mouth, Feeling of numbness and burning sensation in the plasma and soles.

COMPLICATIONS: Cardiovascular diseases, retinopathy, nephropathy, kidney failure, liver damage, slow healing, skin conditions and Hearing impairment

TREATMENT:

Types of Drug	How they work	Example(s)
Alpha-glycosidase inhibitor	Slow your body's breakdown of sugars and starchy foods	Acarbose (percose) and miglitol (Glyset)
Biguanides	Reduce the amount of glucose your liver makes	Metformin (glucophase)
DPP-4 inhibitors	Improve your blood sugar without making it drop too low	Linagliptin (Tradjenta), saxagliptin(Onglyza), and sitagliptin(Januvia)
Glucagon-like peptides	Changes the way your body produces insulin	Dulaglutide(Trulicity), eventide(Byetta),and liraglutide(Victoza)
Meglinides	Stimulate your pancreas to release more insulin	Nateglinide(Starlix)and repaglnide(Prandin)
SGLT2 inhibitors	Release more glucose into the urine	Canagliflozin (Invokana) and dapagliflozi (Farxiga)
Sulfonylureas	Stimulate your pancreas to release more insulin	Glipenclamide, Glyburide Diabeta, Glynase, glipizide (Glucotrol), and glimeperide (Amaryl)
Thiazolidinediones	Help insulin work better	Pioglitazone (Actos) and rosiglitazone(Aavandia)



PANCREAS
ORGAN: The pancreas is an organ located behind the lower part of the stomach, in front of the spine and plays an important part in diabetes. The pancreas is the organ which produces insulin, one the main hormones that helps to regulate blood glucose levels. The pancreas plays a part in two different organ systems, the endocrine system and the exocrine system. The endocrine system includes all the organs which produce hormones, chemicals which are delivered via the blood to help regulate our mood, growth, metabolism and reproduction.Anti diabetic activity of plants: 1.Brosimum alicastrum: Brosimum alicastrum Sw. Is a multi-reason animal categories and it\'s far predominant in a couple of American tropical timberlands In Western Mexico, woods governed through B. Alicastrum, alluded to as mojoteras, had been drastically changed. The current artistic creation desires to convey essential components for the renewed introduction of mojoteras inside the Sierra de Manantlán Biosphere Hold. The question we presented changed into: Are there nursing species for the renewed introduction of B. Alicastrum seedlings in auxiliary tropical dry backwoods? 100 arbitrarily chose 2-year-old B. Alicastrum seedlings were planted in 5 medicines: underneath the overlaying of eighty people having a place with four animal varieties (Tabebuia chrysantha (Jacq.) G. Nicolson, Thouinia serrata Radlk, Acacia macilenta Rose and Acalypha cincta Muell. Arg.) and 20 on open ground. Following 1 a year, the endurance of Brosimum alicastrum seedlings transformed into strikingly selective a large number of the cures (G 2= 12.11, P< zero.05). The medicines with the best large B. Alicastrum seedling endurance rate have been under the covering of Acalypha cincta and Thouinia serrata (55 and 40%, separately), though underneath the covering of Acacia macilenta, Tabebuia chrysantha and on open floor, the endurance rate turned into the base (< 5%).2. Pulicaria dysentrica: Pulicaria dysenterica, the normal name fleabane, or, in North America, glade bogus fleabane, is a types of fleabane in the daisy own family.It is nearby to Europe and western Asia in which it fills in a consequence of natural surroundings beginning from semi-dry Mediterranean forests to wetter circumstances. Pulicaria dysenterica is lasting and may shape thick bunch of verdure, spreading by its foundations. It vegetation at its generally top of around 60 centimetres (2.0 feet). Leaves are on the other hand orchestrated and fasten the stem, which itself contains a pungent astringent fluid. The yellow inflorescences are normally made out of a conspicuous focus of 40–100 plate florets encompassed by 20–30 slim, pistillate beam florets. When putting seed the blossom heads reflex.

3. REVIEW OF DRUG: I: Introduction of plant-1

NAME OF PLANT**:**PULICARIA DYSENTERICA – PLANT (a) DESCRIPTION: The ethereal pieces of pulicaria dysenterica is gathered during the blooming stage from two distinct areas, gathered get-togethers plant grows up to 100cm tall, with yellow blossoms, filling in moist spots and broadly spread in Europe, Iraq, Iran, Pakistan, Afghanistan CHEMICAL COMPOSITION: The compound synthesis of the flying parts oil of Pulicariadysenterica. Bern. was examined by GC and GC/MS. Oils hydro distilled from tests assembled from two interesting regions in Greece were found to have minor compositional differences yet critical assortment in the levels of specific parts. 54 fragments were perceived addressing 80.5% (model An) and 72.6% (model B) of the full scale oils. The essential parts in test A were (Z)- nerolidol (11.2%), caryophyllene oxide (9.1%) and (E)- nerolidol (6.6%), while those of test B were β-caryophyllene (12.8%), caryophyllene oxide (12.8%) and (E)- nerolidol (6.9%).Phytochemicals: Alkaloids, flavonoids, steroids, saponins, tannins, glycosides, and reducingsugar.TAXONOMY:Kingdom:plantae,Class :Equisetopsida,Order:AsteralesFamily:Asteraceae, Genus:Pulicaria, Species:P. dysenterica, Commonname-Fleabane Vernacular names: 1.conyza (butterweed / horseweed) 2. Erigeron (Astraea) 3. Inula (yellow heads. Inuleae)4. Pulicaria (Inuleae) 5. Vernonia (ironweed) 6. Pluchea (camphor weed) LOCATION: Europe, Iraq, Iran, Pakistan, Afghanistan **Therapeutic uses:** Used as anti-parasitic, antibacterialAntimicrobial – leaves, rootAntioxidant – quercetin, thymus and camferolAstringent – leaves Respiratory – flowersCongested cough – flowers The entire plant has recuperating, appealing (because of the contained unpleasant specialists) and against diarrheal properties. It is utilized for treatment of loose bowels

(squashed leaves), too to invigorate the assimilation. Additionally, it is suggested as home grown tea or plant\'s concentrate against hack and bronchitis, as it has expectorant and narcotic properties. Absorption, The runs, Diabetes , Diuretics , Loss of hunger, Kidneys and Bladder, Feminine issues, Weight, Post pregnancy dying, Drain, Kidney energizer. Subsequently, in light of the above restorative employments of pulicaria dysenterica, we propose to explore the Counter diabetic movement of the plant instreptozotocin initiated diabetic rodents

Plant name: BROSIMUM ALICASTRUM - PLANT-(b) Description: The plant is gathered get-togethers The tree can grow up to 45 m (130 ft) in tallness and up to 1.5 m in width. This tree is found on the west shoreline of focal Mexico and in southern Mexico (Yucatán, Campeche), Guatemala, El Salvador, the Caribbean, and the Amazon. Enormous stands happen in sodden swamp tropical timberlands at300–2000 m height (particularly 125–800 m), in moist regions with precipitation of 600–2000 mm, and normal temperatures of 24 °C (75 °F).

CHEMICAL COMPOSITION: The purpose of the prevailing take a look at became to investigate the antioxidant activity and general phenolic content material (TPC) of Maya nut (Brosimum alicastrum) in comparison with commercially to be had nuts (i.e. Walnut, almond, and peanut). Results indicated that Maya nut had the very best TPCs among these nuts. Maya nut also possessed strong 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and a couple of.2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging sports and ferric reducing antioxidant power (FRAP) ($p < zero.05$) as compared to walnut, almond, and peanut. Five phenolic acids (Gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, and p-coumaric acid) and one flavonoid ((-)-epicatechin) had been recognized and the phenolic content ranged from 6.5 to 326.2 µg/gPhytochemicals: Flavonoids, alkaloids, glycosides, steroids, saponinns Taxonomy:Kingdom: plantaeFamily- MoraceaeGenera – Mulberry/ figs Species- B.A. alicastrumCommon name- breadnut/ Maya nutVernacular names: English: Ramon treeSpanish: masico Belize: capomoCosta Rica: lechoso, ojoche, Ramon, ramonblancoCuba: guaimaroGermany: Brotnussbau mGuatemala: ujushteHonduras: masica; masico; oxItaly: campoJamaica: breadnutMexico: ox; talcoitePanama: berbaTrinidad and Tobago: moussara,Venezuela: barimison, Location: west coast of central Mexico and in southern Mexico (Yucatán, Campeche), Guatemala, El Salvador, the Caribbean,

Therapeutic uses:

- There is a belief in Yucatan that if the seeds are eaten by using nursing girls the waft of milk is accelerated.
- The latex is applied immediately on sores inside the mouth and different parts of the body for healing
- The latex is blended with water, warmed and inebriated as a remedy for dry coughs and for itchy sore throat.
- The seeds and the fruit of the tree were utilized in conventional Mayan medicine for the treatment of menopause and osteoporosis, and are notion to have hormone-like activities.
- Therefore, primarily based on the above medicinal uses of brosimumalicastrum we propose to analyze the Anti- diabetic pastime of the plant in streptozotocin induced diabetic rats and also to establish the headaches including Anti-hyperlipidimia and anti-oxidants.

4. Aim: The aim of the present study is to evaluate the leaves extract of pulicaria dysenterica and brosimum alicastrum for antidiabetic activity and possible complications of hyperlipidemia and antioxidant Objectives:

1.Collection and Authentification of plants 2.Plants extraction by Maceration technique 3.Phytochemical screening of plants 4.Confirmation of chemical constituents 5.Screening of anti-diabetic activities 6.Biochemical estimation such as α- Amylase Inhibition assay, α -Glycosidase Inhibition assay, Blood glucose levels, LDL, VLDL, HDL, TRG, Total cholesterol,

6. MATERIALS AND METHODS:

6.1 Collection and Authentication of plants material:

The leaves of pulicaria dysenterica and brosimum alicastrum are collected and authenticated by botanist DR Madhav shetty DEPARTMENT Of Botany **Material Required**: Glass jars, Ethanol, Powdered plants, Spatulas, Aluminium foil, Standard drug.

6.2 ETHANOLIC EXTRACTS PREPARATION: The extract is made by maceration process. The extract is macerated with Ethanol for 7days and after that shifted. The filtrate is evaporated to get dried extract.

6.3 MACERATION: The plant powdered is kept in contact with the dissolvable ethanol in proportion of 1:2 and overwhelming mixing is carried out, after 7days, the extract is shifted out for drying.

CHEMICALS AND REAGENTS:

1. Normal saline: (0.9%w/v) – used as solvent to dissolve the test and standard drugs
2. Ethanol 99% v/v -- preparation of plants extract
3. Glibenclamide -- standard drug for anti diabetic activity
4. Streptozotocin - Toxic control to induce diabetes

6.4 PHYTOCHEMICAL EVALUATION:

Phyto-chemical tests done on the plants extracts are for identifying secondary metabolites such as glycosides, alkaloids, saponins, tannins, and flavonoids.

1. *Tannins: 200mg of plant extract was boiled in 10ml of distilled H2O & few drops of FeCl3 were added to filtrate, If a blue-black precipitate appears it indicates the presence of Tannins.*

2. *Alkaloids:* 200mg of plant extract was boiled in 10ml of methanol and then filter it. To the filtrate add 1%HCL followed by 6 drops of dragondroff`s reagent, the brownish-red precipitate was taken as the evidence for the presence of alkaloids

3. *Saponnins :(frothing test) 5ml distilled water was added to 200mg of plant extract. 0.5ml filtrate was diluted to 5ml distilled water & shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins*

4. *Glycosides (Keller-Killiani test):2ml of filtrate was treated with 1ml glacial acetic acid containing few drops of FeCl3; Conc.H2SO4 was added to the above mixture giving green-blue color depicting the positive results for the presence of glycosides.*

5. *Steroids (Liebermann- Bur chard reaction):200mg plant extract was added in 10ml of chloroform, Acetic anhydrate was a deed in the ratio of 1:1 which resulted into the formation of blue-green ring pointing towards the presence of steroids.*

6. *Flavonoids: To the aqueous filtrate 5ml of dilute ammonia solution was added, followed by concentrated H2SO4. A yellow coloration indicates the presence of flavonoids.*

6.5 GCMS ANALYSIS: we are using the technique of gcms to find out various chemical constituent on the plant.

6.6 EXPERIMENTAL ANIMALS: The exploratory examination was led at shadan institute of medical sciences, Khairtabad, Hyderabad. White Albino Wister Rodents weighing 150- 200 gm were accustomed for 14 days in animal house of shadan organization of medical sciences. The chosen animals are housed in wire mesh, with aerated cages through confines agreeable temperature (25+ 5C) and 12 hours light\dark cycle. Free admittance to water and feed is provided as per the OECD guidelines. Permission and approval ofanimal studies was obtained from IAEC, after submission of form B and protocol no:

Induction of diabetes: Rats were weighed and diabetes was induced by intra-peritoneal injection of streptozotocin (STZ) in a single dose of 45mg/kg body weight, dissolve in Saline buffer (PH). After 48 hrs, animals showing fasting blood glucose levels at 200mg/DL and above are selected for the study. Fasting glucose level was determined in all the six groups 72 hrs after induction of diabetes and continually measured weekly afterwards at 7th, 14th, and 21st day with glucometer test strips.

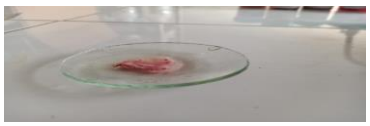
Streptozotocin induced diabetic rat: After inducing streptozotocin the glucose and feed is given to the rats and then after 48hr the BGL is examined by tail pricking method .Animals will be divided into 9 groups of 6 animals each pertaining to the grouping. Now the rats are treated with standard and plants extracts till 21 days Then again BGL is examined and Animal will be anesthetized (CO), dissected and pancreas will be histopathologically examined. **Grouping of animals**: The animals are divided equally into nine groups consisting of six in each group No. of Animals required: - 54 Male Wister Rats.

GROUPS	TREATMENT	NO.OF RATS
GROUP-1	Distilled water(5ml/kg.p.o), for 21 days	6
GROUP-2	Streptozotocin + water (5ml/kg,p.o), once daily for 21 days	6
GROUP-3	Glipenclamied (120mg/kg.p.o), once daily for 21days	6
GROUP-4	Plant A (200mg/kg,p.o), once daily for 21days	6
GROUP-5	Plant B(200mg/kg,p.o), once daily for 21days	6
GROUP-6	Plant A+ Plant B(200mg/kg,p.o), once daily for 21days	6

Completion of experimental period, i.e.; on 28th day, after providing their respective treatments, blood samples will be collected from each rat of every group by retro group and tested for fasting blood glucose, Insulin, and haemoglobin results.

Blood Collection: - RETRO ORBITAL PUNCTURE


Pancreas sample collection


Zoomed in view of the pancreas sample collected


Blood glucose meter


Isolation of pancreas


Intra-peritoneal injection to Albino rats


Feeding of drugs with oral gavages

6.7Acute oral toxicity studies:

The experimental study is performed in white male Albino sister rats, according to the OECD guidelines no.423 with both the extracts.

Procedure: Invitro methods:1. Assay of amylase inhibition: The assay was carried out following the standard protocol with slight modifications. Starch azure (2 mg) was suspended in 0.2ml of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M CaCl2 (substrate solution). The tubes containing substrate solution were boiled for 5 min and then pre incubated at 37°C for 5 min. Ethanol extract of P. amarus was dissolved in DMSO in order to obtain concentrations of 10, 20, 40, 60, 80, and 100ug/ml. Then, 0.2 ml of plant extract of particular concentration was added to the tube containing the substrate solution. In addition, 0.1 ml of porcine pancreatic amylase in Tris–HCl buffer (2units/ml) was added to the tube containing the plant extract and substrate solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer (Perkin Elmer Lambda 25 UV–VIS spectrophotometer). Same procedure was followed for other plants extracts (chloroform and hexane) to test their α-amylase inhibitory effects. Acarbose, a known α-amylase inhibitor was used as a standard drug. The experiments were repeated thrice.

2. Inhibition of α-glycosidase activity: The inhibition of *α*-glycosidase activity turned into

determined the usage of the changed published technique. 1mg of α-glycosidase (Saccharomyces cerevisiae, Sigma-Aldrich, USA) was dissolved in 100 ml of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin (Merck, German). The response combination consisting 10µl of sample at various concentrations (0.52 to 3µg/ml) become premixed with 490µl phosphate buffer pH 6.Eight and 250µl of 5m M-nitro phenyl α-D-glucopyranoside (Sigma-Aldrich, Switzerland). After pre incubating at 37°C for 5 min, 250µl α-glycosidase (0.15unit/ml) become added and incubated at 37°C for 15 min. The reaction became terminated via the addition of 2000µl Na2CO3 200 mm. α-glucosidase interests became determined spectrophotometrically at 400 nm on spectrophotometer UV-Vis (Shimadzu 265, Jepang) with the aid of measuring the quantity of *p*-nitro phenol released from p-NPG. Acarbose became used as high-quality manipulate of α-glycosidase inhibitor. The awareness of the extract required to inhibit 50% of α-glycosidase hobby under the assay situations became defined as the IC50 price.

INVIVO METHODS:1. Blood glucose level (BGL): A blood sugar stage much less than a hundred and forty mg/dl (7.8mmol/l) is regular. A studying of extra than 2 hundred mg/dl (11.1mmol/l) after two hours indicates diabetes. A reading between one hundred forty and 199 mg/dl (7.8mmol/l and 11.0mmol/l) shows pre diabetes**. 2.Total cholesterol level:** Target LDL cholesterol levels for adults with diabetes are <100 mg/dl (2.60mmol/l); HDL cholesterol levels are >40 mg/dl (1.02mmol/l); and triglyceride tiers are <one hundred fifty mg/dl (1.7mmol/l).In women, who generally tend to have better HDL cholesterol levels than men, an HDL intention 10 mg/dl better may be suitable.**3.Triglycerides (TRG):**Triglycerides are fat molecules that make up most of your body fat and the fat found in food. Along with cholesterol, they are one of the lipids that circulate in your blood. The medical term for having elevated levels of triglycerides is hypertriglyceridemia. In fasting laboratory tests, a normal triglyceride level is below 150 milligrams per decilitre (mg/dl). Borderline high is 150 to 199 mg/dl. High is considered 200 to 499 mg/dl. Very high is over 500 mg/dl. **4. Serum glutamic-oxaloacetic transaminase (SGOT):**The everyday range of an SGOT take a look at is generally among eight and forty five gadgets in line with litre of serum. In fashionable, men may also clearly have higher quantities of AST inside the blood. A score above 50 for men and forty five for ladies is high and can suggest damage **5. Serum alkaline phosphate (ALP):**SGPT exists predominantly in the liver and leaks into the bloodstream while produced in greater. The SGPT normal variety is set 7 to fifty six gadgets in line with litre of blood serum. Thus, very excessive degree of SGPT inside the blood can be an illustration of harm or problems associated with the liver. Certain diseases like cirrhosis and hepatitis boost the blood serum SGPT degrees, so do particular medicines which includes statin used to decrease cholesterol .**Histopathology of pancreas:**After dissection of animals, pancreas will be isolated for the histopathological investigation.**Statistical analysis**: Records could be expressed as mean -+SEM. p<0.05 can be set at considerable degree. ANOVA observed by way of Turkey's Multiple Comparision Test.

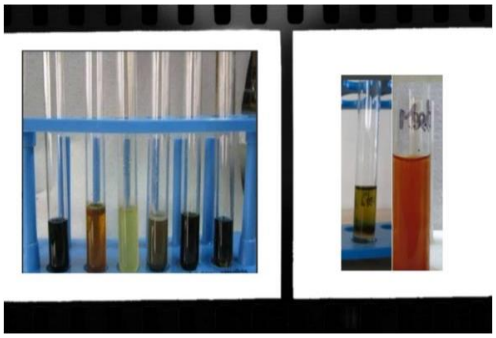
7) RESULTS:

1 .Maceration:

We accrued the ethanolic extract of plant A & plant B by maceration.

2. Phytochemical analysis:

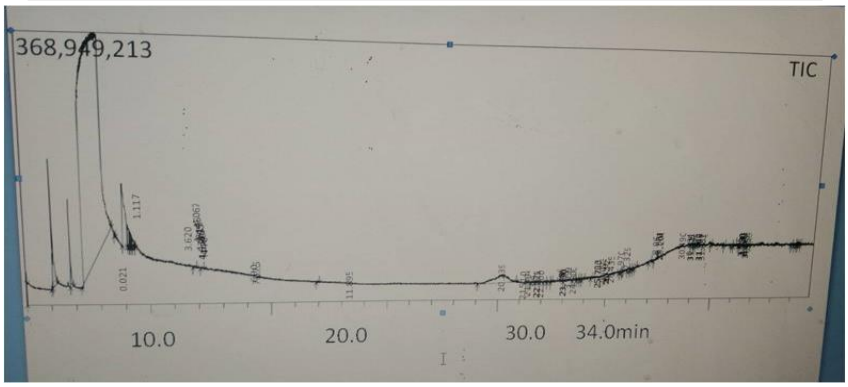
Active principles	Tests	Plant-1	Plant-2
Tannins	FeCl3	-	+
Glycosides	Fehling`s test	-	+
Alkaloids	Wagner`s test	-	+
Saponinns	Frothing test	-	+
Steroids	Salkowoski test	-	+
Flavonoids	NaOH	+	+
Reducing sugars	Benedict`s test	+	+



Key = + is present -is negative **Test tubes sample showing phyto-chemical results (1.Tannins, 2.Alkaloids, 3.Flavonoids, 4.Saponinns, 5.glycosides, 6.Steroids, 7.Reducing sugars.)**

3. GCMS ANALYSIS: PLANT-1

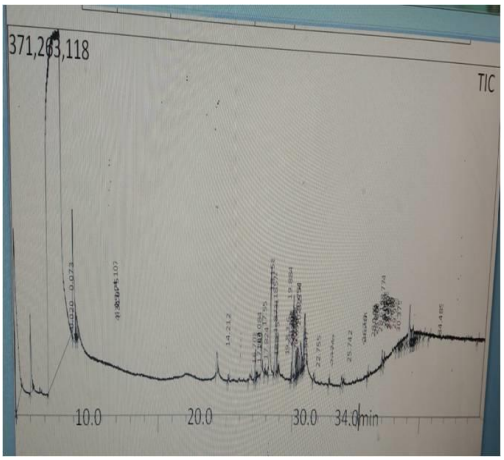
S.NO	Retention time	Chemical constituents	Area %	Uses
1	18.73	Octasiloxane	0.44	Antioxidant, antidiabetes,antimicrobial
2	26.95	Sarsasapogenin	0.16	Antitumor, antidiabetes,antifungal
3	29.016	2-hydro4methoxybenzoic acid	0.12	Antimicrobial, antidiabetes,antifungal
4	14.212	Diethyl phthalate	0.49	Antidiabetes,lubricants,antimicrobial
5	28.774	neotigogenin	0.81	Hyperlipidemia, hyperglycemia



As the ethanolic plant extract of pulicaria dysentrica contains the above chemical constituents which shows the antidiabetic activity. The graphic figure shows the retention time and area percentage of all the chemical constituents present in the leaf extract of pulicaria dysentrica by GCMS analysis.

PLANT-2:

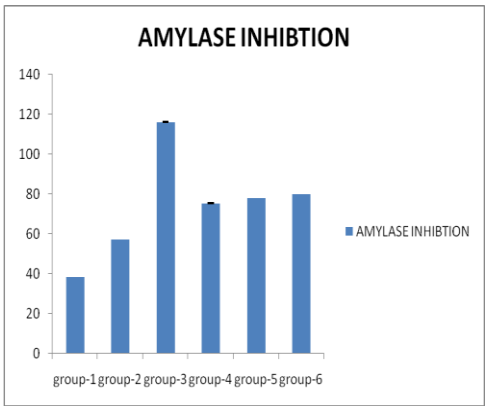
SNO:	Retention time	Chemical constituents	Area%	Uses
1	41.45	Sulfamide	0.67	Antidiabetes,antimicrobial, antiinflammatory
2	31.4	Hydroxycinammic acid	0.12	Antidiabetes,antimicrobial, antiinflammatory
3	27.3	Silicic acid	0.10	Antidiabetes,antimicrobial, antiinflammatory
4	29.1	Eburnamenine	0.08	Anticholenergic, antidepressant,antidiabetes
5	26.1	Heptasiloxane	0.09	Antioxidant ,preservatives, antibacterial



As the ethanolic plant extract of brosimum alicastrum contains the above chemical constituents which shows the antidiabetic activity. The graphic figure shows the retention time and area percentage of all the chemical constituents present in the leaf extract of brosimum alicastrum by GCMSanalysis.

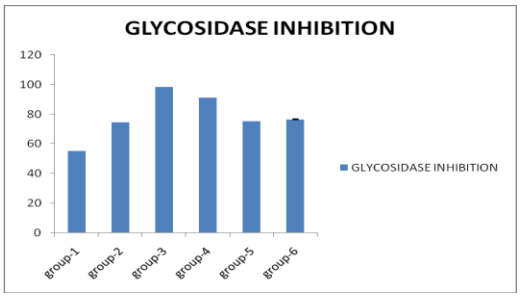
4. INVITRO:

1.α amylase inhibition :



Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,5,6 are found to be more significant

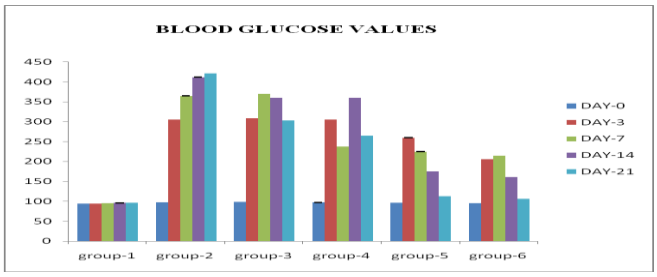
2. Inhibition of α-glycosidase activity:



Data was demonstrated as Mean ± rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,4,6 are found to be more significant

IN-VIVO

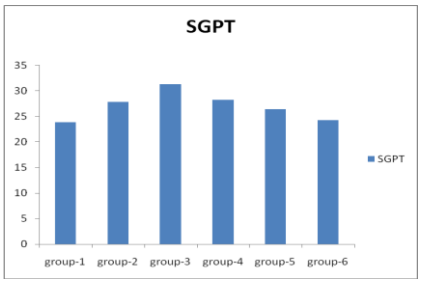
1.BLOOD GLUCOSE LEVELS



Normal healthy rats have fasting glucose levels at 80-100 mg\DL, in this experiment, rats having fasting blood glucose over 200mg\DL or more will be considered diabetic as streptozotocin destroys all pancreatic islets, increasing blood glucose levels up to 300mg\DL. In the first week, glucose levels were reduced in the group which was treated with Plant-1+2 Ethanolic extract of Pulicaria dysentrica and Brosimum Alicastrum the group which was treated with the Standard drug (Streptozotocin), this shows that Ethanolic extract of Plant-2 extract is equally potent with that of standard drug, The group which are treated with Plant 2 ethanolic extract was potent as that of a group that are treated with plant1+2 extract, and the group which are treated

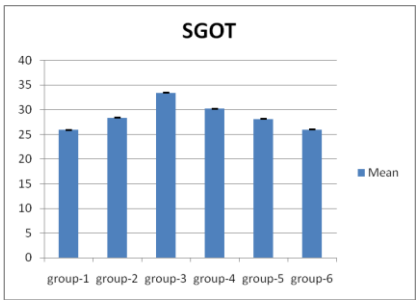
with plant-1 shows less potency than plant-2 and plant1+2, blood glucose levels of normal control group i.e., untreated group is high. This show that the plant-2 is more potent than plant-1 and the combination of both the plant1+2 shows are potent as that of standard drug, this confirms the Anti-diabetic activity of Pulicaria Dysentrica and Brosimum Alicastrum leaf extracts in streptozotocin induced diabetic rats.

2. SGPT VALUES:



Graph7: Effect of EEPD and EEBA on SGPT streptozotocin induced antidiabetc rats

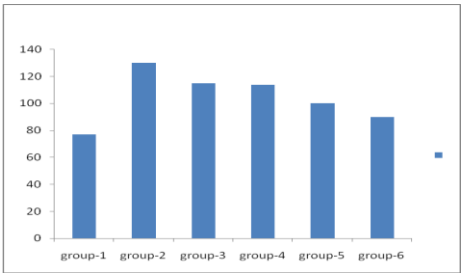
3. SGOT:



Graph 8: Effect of EEPD and EEBA on SGOT streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,5,6 are found to be more significant

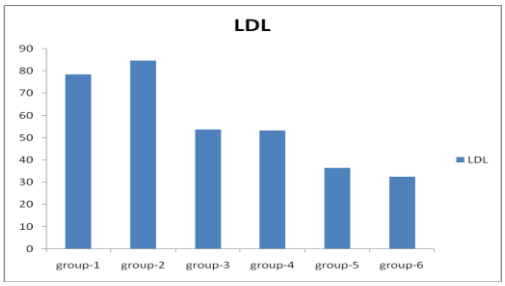
4. TCL (total cholesterol levels)



Graph 9: Effect of EEPD and EEBA on TCL streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,4,6 are found to be more significant

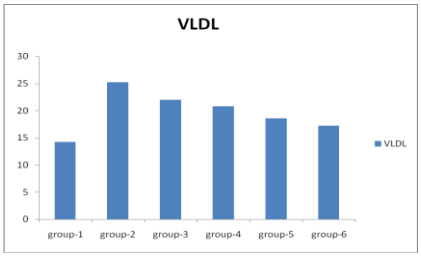
4. LDL:



Graph10: Effect of EEPD and EEBA on
LDL streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,4,6 are found to be more significant

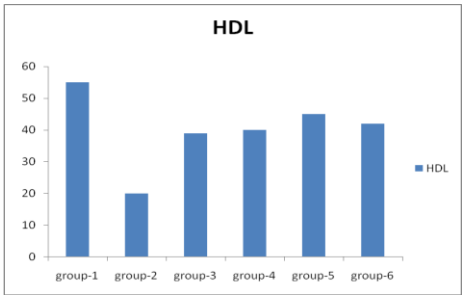
6 VLDL:



Graph11: Effect of EEPD and EEBA on
VLDL streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,4,6 are found to be more significant

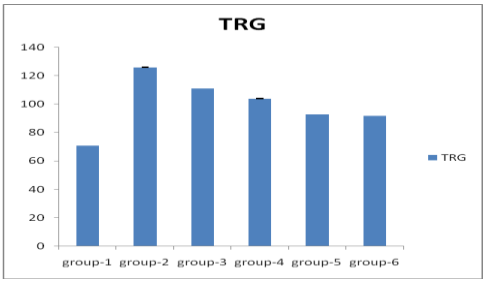
7. HDL:



Graph12: Effect of EEPD and EEBA on
HDL streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,5,6 are found to be more significant

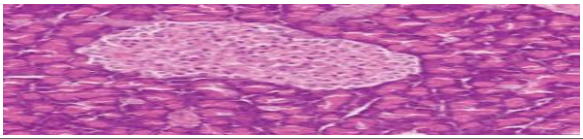
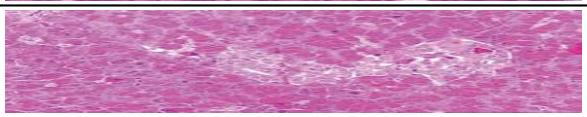
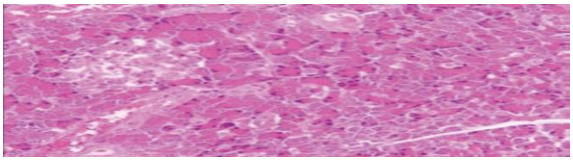
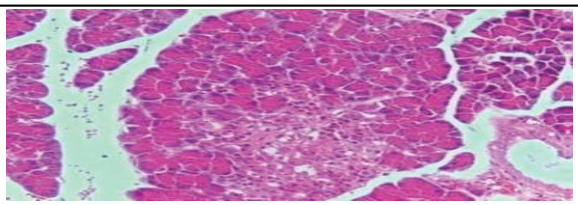
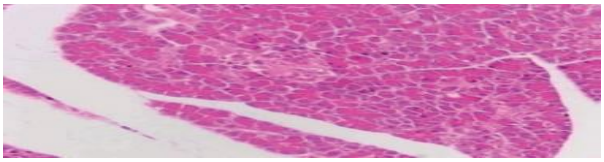
8TRG:



Graph14: Effect of EEPD and EEBA on TRG streptozotocin induced antidiabetc rats

Data was demonstrated as Mean ± S.E.M, n=6 rats in each group =P<0.05>, +P<0.001, +P<0.0001 are considered significant when compared with amylase inhibition control group. Among all the treatment groups, 3,4,6 are found to be more significant

HISTOPATHOLOGY RESULT:

GROUPS	INVESTIGATION
Normal control: Normal Pancreas: Normal rat showing normal acini and normal cellular population in islets of langerhans and absence of both damage to islets and hyperplasia.	
Toxic control: Streptozotocin Diabetic control rat showing damaged islets and reduced islets size.	
Standard control: Glipenclamide Diabetic rats treated with GLB (5mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.	
Ethanolic Plant Extract :Plant-1 Diabetic rat treated with EPL (200mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.	
Ethanolic Plant Extract:Plant-2 Diabetic rat treated with EEM (200mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.	
Ethanolic Plant Extract:Plant1+2 Diabetic rat treated with combination of EEM (100mg/kg) and EPL (100mg/kg) showing restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.	

DISCUSSIONS:

In diabetes, Hyperglycemic condition leads to development of a several damages to the organs like kidneys, pancreas, liver, spleen, heart and in blood vessels of retina (diabetic retinopathy). Natural remedies used in treating diabetes lend use of ayurvedic system of medicine. In this investigation, we mainly focused on antidiabetic properties of ethanolic leaf extracts of Pulicaria dysentrica and Brosimum alicastrum. Diabetes was chemically induced with streptozotocin (45mg/kg) in male Wister rats belonging to all groups other than normal group. After 48hrs, animals showing fasting blood glucose levels at 200mg/dl and above selected for the main study. Fasting glucose levels was determined in all the six groups 48-72hrs after inducing diabetes and continuously measuring weekly once for 21 days with glucometer test strips (Accu-check advanced). Literatures suggest that, streptozotocin destroys all pancreatic islets; thereby increasing blood glucose levels up to 400mg/dl. In the first week, glucose levels were fairly reduced in the group that received higher dose of aqueous extract of brosimum alicastrum almost similar to the group of standard drug. The decreased in fasting glucose levels in the group that received higher dose of pulicaria dysentrica. This shows that plant 1 is equally potent with that of standard drug. Blood glucose levels of control group (untreated group) remained high. In our study, we have recorded similar observations that confirm the antidiabetic activity of plant-1 extract against streptozotocin induced diabetic rats. There are experimental studies have been established to determined the chemical compounds responsible for the activity. In an attempt to test phytoconstituents and extract of plant-1&2 the purified ligands of plants extract obtained from this plant were evaluated in streptozotocin induced model. Therefore, playing a key role in glucose haemostatic. These plant extract contain compounds like flavonoids, glycosides, alkaloids, tannins, & saponins, that are responsible for antidiabetic activity. Insulin stimulates and activates glucokinase in the pancreas. This enzyme is an important regulator of glucose storage and disposes. In the present study, the glucokinase activity was decreased in streptozotocin induced diabetic rats which may be due to insulin deficiency. Pulicaria dysentrica and brosimum alicastrum leaf extracts or streptozotocin stimulate insulin secretion by elevating the levels of glucokinase thereby increasing utilization of glucose in turn decreasing blood sugar level.Therefore, as per the data, our investigation proves the antidiabetic property of pulicaria dysentrica and brosimum alicastrum plant extract.

CONCLUSION:

The present observation suggest that leaf extract of pulicaria dysentrica and brosimum alicastrum exhibited Anti-Diabetic activity on streptozotocin induced diabetic male Wister rats, which may be by stimulating pancreatic enzymes involved in the process and to reduce the presence of flavonoids, alkaloids, glycosides, and other constituents in the leaves which could potentiate action combined or individually. Hence, comprehensive further pharmacological investigation should be carried out to study individual active compounds and their mechanism of action. Results henceforth prove that it is worth having studies on usefulness of the pulicaria dysentrica and brosimum alicastrum leafs in anti diabetes. These statements confirm plant 2 was less potent then plant 1 and the combination of both the plants is more potent than individual plants.

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