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Prepration & Evaluation Of Polyherbal Suspension For Anti-Diabetic Activity

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Abstract:
In the present study the antihyperglycemic properties of the specially formulate polyherbal formulation in Streptozotocin induced diabetic rats was determined. Diabetes was induced in Albino rats by administration of streptozotocin. The formulation i.e Polyherbal suspension was administered to diabetes induced rats for a period of 45 days, which possess better effect. Additional biochemical parameters such as serum cholesterol, triglycerides, HDL-cholesterol, LDL cholesterol levels were also measured at the ending of study. After verify the antidiabetic property of Polyherbal suspension on blood glucose was observed the finest one, in order to justify it we have to check its oxidative parameter , and LPO, Which enzyme indicate its oxidative stress. In the present study poly herbal extracts of antidiabetic activity of Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China are prepared and converted into suspension using organic solvents. Those polyherbal suspensions were evaluated. From the above outcome it is concluded that the formulation 200 mg/kg on blood glucose possesses significant antidiabetic effects in streptozotocin induced diabetic rats. By through analytical justification i.e chromatographic separation, HPLC techniques, Spectroscopic technique the responsible phytoconstituent for physicochemical property was more justified.
Key Words: *Antidiabetic Herbs, antidiabetic activity of Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China.*

I. INTRODUCTION OF ANTI-DIABETIC HERBS

For most herbs, the specific ingredient that causes a therapeutic effect is not known. Whole herbs contain many ingredients, and it is likely that they work together to produce the desired medicinal effect. The type of environment in which a plant grew will affect its components, as will how and when it was harvested and processed. Evaluating the hypoglycemic ability of medicinal plants has therefore become essential. In the current study, antidiabetic activity is being investigated, a polyherbal extract made from an equal mixture of Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China, fruit coat is prepared, polyherbal Formulations are made, evaluated, and their stability is being studied. Unani polyherbal formulations like Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China, and the like are used in treatment of Diabetes mellitus. Among the Unani anti-diabetic formulations, **"**Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China,**"** is a reputed and popular polyherbal formulation scientifically under explored. This formulation suffers from patient's non-compliance because of cumbersome dosage form, instability, difficulty in dose selection and administration. Sophisticated, modern instruments were used as an advanced tool in phyto pharmaceutical evaluation of the selected polyherbal formulations so as to prescribe the quality standards for better therapeutic efficacy. The toxicological evaluation of herbal drug ingredients like determination of pesticide residues, heavy metal contamination and microbial contamination and their formulation Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China, for acute toxicity studies using recent advanced analytical tools have been carried out in keen interest of uplifting the herbal drug to the global markets. The extract of Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China, exhibited significant anti-hyperglycemic activity in Streptozotocin (STZ) induced diabetic rats. This extract showed improvement in parameters like body weight, food consumption, organ weight and biochemical parameters and might be of great valuable in diabetic treatment.

II. Collection and authentication of plant material

In the present study, the Amla, Baheda, Jamun, Karela, Nut Tree, Smilex China was collected from Yucca Enterprises wadala Mumbai.

The collected parts were dried under shade at room temperature and powdered to coarse consistency in grinder mill. The powder was passed through 40 # mesh particle size and stored in an airtight container at room temperature.

Table 1 Chemical and Equipments

S.No.	Materials		Supplier
1.	Streptozotocin	Pharma	Spectrochem Pvt. Ltd., Mumbai, India
	Glimipride	Pharma	Aventis Pharma Ltd., Verna, Goa
3.	Dextrose	Pharma	Emkay Labs, Mumbai, India
4.	Tween 80	Pharma	S. D. Fine-chem limited, Mumbai, India
5.	Anesthetic Ether	A.R.	Ozone International, Mumbai, India
6.	Accu-chek® Active Glucometer	Pharma	Roche Diagnostic Corporation, Germany
7.	Blood gluco-strips	Pharma	Roche Diagnostic Pvt. Ltd., Mumbai, India
8.	Triton –WR 1339	Pharma	S D Fine chemicals, Mumbai, India
9.	Simvastatin	Pharma	Dr. Reddy's Laboratories, Hyderabad

All other chemicals and reagents used were of analytical grade.

A. Physico-Chemical Evaluation of Crude Drugs

All the crude drugs were subjected to physical and chemical evaluation for different parameters. Physical evaluation is the primary step adopted in the identification and standardization of crude drugs. It helps in the determination of adulterants and validates the authenticity of crude drug. It is also the primary step adopted in the identification of chemical constituents and standardization of crude drug.

B. Extractive values

- **Determination of alcohol- soluble extractive**

Macerate 5g of the air-dried coarsely powdered crude drug with 100ml ethanol of the specified strength in closed flask for 24hrs, shaking frequently during the first 6hrs and allowing standing for next 18hrs. Filter rapidly taking precautions against loss of ethanol, evaporate 25ml of the filtrate to dryness in tarred, flat bottomed, shallow dish and dry at 105^0C to constant weight. Calculate the percentage of ethanol soluble extractive with reference to the air-dried drug.

- **Determination of Water-soluble extractive**

Proceed as directed for the determination of alcohol soluble extractive, using chloroform water instead of alcohol.

- **Determination of Chloroform-soluble extractive**

Proceed as directed for the determination of alcohol soluble extractive, using chloroform instead of alcohol.

- **Determination of Petroleum ether-soluble extractive**

Proceed as directed for the determination of alcohol soluble extractive, using petroleum ether instead of alcohol.

C. Loss on drying

Loss on drying is the loss of mass expressed as percent m/m. About 5-6g of drug powder is accurately weighed in a Petri dish and kept in a hot-air oven maintained at 110^0C for four hours. After cooling in dessicator, the loss in weight was recorded in each case. This procedure was repeated till the constant weight was obtained.

Loss on drying (%) = loss in weight X 100/ W

W= weight of the drugs in grams.

D. Ash Values Total ash

Method 1: Take about 2 to 3g, accurately weighed powdered drug in a tarred platinum or silica dish previously ignited and weighed. Scatter the powdered drug on the bottom of the dish. Incinerate by gradually increasing the heat, not exceeding dull red heat until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, add the filtrate, evaporate the residue and ignite at low temperature. Calculate the percentage of each with reference to the air-dried drug.

Method 2: Heat a silica or platinum crucible to redness for 30min, allow cooling in desiccators and

weighing unless otherwise prescribed, evenly distribute 1g of the substance or powdered vegetable drug to be examined in the crucible. Dry at 100^{0}C –105^{0}C for 1hr and ignite to constant mass in a muffle furnace at 600 ± 25^{0}C, allowing the crucible to cool in a dessicator after each ignition. Flames should not be produced at anytime during the procedure. If after prolonged ignition, the ash still contains black particles, take up with hot water, filter through ashless filter paper, combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant mass.

E. Acid-insoluble ash

The ash was boiled for 10 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected in a crucible. It was washed with hot water, ignited and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

F. Water-soluble ash

The total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected in crucible. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

G. Fluorescence analysis

Many crude drugs show the fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not used as it is usually unreliable due to the weakness of the fluorescent effect (Umbelliferone test used for galbanum and asafoetida is, however an exception). Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation of definite wavelength. Several crude drugs show characteristic fluorescence for their evaluation.

H. Determination of foreign organic matter

The parts of the organ or organs other than those named in the definition and description of the drug are defined as foreign organic matter. The maximum limit for the foreign organic matter is defined in the monograph of crude drugs. If it exceeds the limits, deterioration in quality of the drug takes place. The limit for foreign organic matter is specially mentioned for natural drugs of vegetable origin in their respective monographs.

III. Observations of Physical Evaluation:

Table No. 2 Physical Test of Crude Drugs

Crude drugs	Physical Test			
	Nature	Colour	Odour	Taste
Amla	Coarse powder	Yellowish	Characteristic	Astringent
Baheda	Coarse powder	Yellowish	Characteristic	Bitter
Jamun	Coarse powder	brown	Slight	Bitter
Karela,	Coarse powder	Green	Faint	Bitter
Nut Tree	Coarse powder	brown	Characteristic	bitter
Smilax china	Coarse powder	Brown	Characteristic	Agreeable

Table No. 3 Extractive Values*

Crude drugs	Pet-ether % w/w	Chloroform % w/w	Alcohol % w/w	Aqueous % w/w
Amla	1.79	4.63	18.54	20.86
Baheda	1.46	3.62	14.65	18.45
Jamun	2.15	4.12	14.23	17.50
Karela,	1.20	4.95	22.56	20.78

Nut Tree	1.2	2.65	19.7	17.54
Smilax china	0.74	4.25	16.31	17.07

Table No. 4 Loss on Drying and Foreign Organic Matter

Crude drugs	Loss on drying (% w/w)*	Foreign matter (% w/w)*
Amla (Emblica officinalis.	6.35	1.75
Baheda (Terminalia bellerica)	4.37	1.89
Jamun (Syzygium cumini)	6.24	1.83
Karela, (Momordica charantia)	8.02	1.67
Nut Tree (Strychnos potatorum)	4.5	1.96
Smilax china (Acorous calamus)	7.20	1.04

Table No. 5 Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Crude drugs	Total Ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
Amla	6.35	3.54	1.50
Baheda	9.76	2.47	0.71
Jamun	7.25	2.85	0.98
Karela,	4.90	3.14	0.89
Nut Tree	3.58	1.58	0.64
Smilax china	4.20	1.79	0.68

Table No. 6 Fluorescence Analysis

Reagents	Observation (Colour developed in daylight)					
	Amla	Baheda	Jamun,	Karela,	Nut Tree	Smilax china
Conc. H_2SO_4	Violet	Dark brown	Brownish Black	Brownish Black	Brown	Brownish Black
Glacial acetic acid	Yellow	-	Pale brown	Green	-	Reddish brown
5% $FeCl_3$	Brown	Green	Green	-	Green	Green
Ammonia solution	Pale buff	Pale green	Greenish brown	Green	Pale green	Greenish brown
Acetic acid+ $FeCl_3$+ConcH_2SO_4	Yellowish brown	Greenish brown	-	Yellowish brown	Brown	Dark brown
Iodine	Reddish brown	Reddish brown	Reddish brown	DarkYellow brown	Reddish brown	Reddish brown

Acetic acid + Conc.H_2SO_4	Brown	Pale yellow	Pale brown	Green	Yellow	Brown
Picric acid	Yellow	Yellowish green	Yellowish brown	Yellowish green	Yellowish green	Yellowish brown
1N HCl	Pale yellow	Pale brown	Pale brown	~	Brown	Reddish brown
10% NaOH + 10% CH3COOPb	Yellowish brown	~	Greenish brown	~	Brown	Brown
HNO_3+NH3 sol.	~	Greenish brown	Greenish brown	Pale green	Pale green	Dark brown
10% NaOH + $CuSO_4$	~	Dark blue ppt	Blue ppt.	Blue ppt.	Blue ppt	Bluish green ppt
1N NaOH	~	Pale brown	Brown	~~~~	Brown	Dark green

IV. Extraction and Phytochemical Evaluation of Crude Drugs

- **Preparation of pet-ether (60-80^0) extract**

The fresh air-dried, powered crude drug was extracted with pet ether (60-80^{0C}) at room temperature for seven days in a 2000-5000 ml conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites. The dried extract was stored carefully for phytochemical investigation and development of antidiabetic formulation.

- **Preparation of chloroform extract**

Proceed as directed for the preparation of pet-ether extract using chloroform as a solvent.

- **Preparation of alcoholic extract**

Proceed as directed for the preparation of pet-ether extract using alcohol as a solvent.

- **Preparation of aqueous extract**

Proceed as directed for the preparation of pet-ether extract using chloroform water I. P. as a solvent.

V. Qualitative Phytochemical Investigation [3-5]

The various extracts were subjected to phytochemical investigation by following standard procedures as follows:

1. **Test for Alkaloids**

The test solution was prepared by dissolving extracts in dilute hydrochloric acid.

a) Mayer's test: Test solution with Mayer's reagent (Potassium mercuric iodide) gives cream color precipitate.

b) Dragendorff's test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) shows orange brown precipitate.

c) Wagner's test (solution of iodine in potassium iodide): Treat the test solution with Wagner's reagent, reddish brown precipitate forms.

d) Hager's test (Saturated solution of Picric acid): Treat the test solution with Hager's reagent, yellow colour precipitate occurs.

2. **Test for Glycosides**

The extract is tested for free sugars. After complete removal of sugars, the extract is hydrolyzed with dilute mineral acid and then tested for the glycone and the aglycone moieties.

Test for Cardiac Glycosides

a) Liberman- Burchardt's test
b) Keller-killaini test
c) Raymond's test
d) Baljet test

Test for Cyanogenetic Glycosides

To one gram of powdered drug moistened previously in a test tube, suspend a piece of sodium picrate paper above the drug by trapping the top edge between the cork and the tube wall. Allow standing for

thirty minutes, the evolution of hydro cyanic acid turns the paper brick red (sodium isopurpurate).

Test for Anthraquinone Glycosides

Borntrager's test: Powdered drug is boiled with dilute sulphuric acid and filtered. The filtrate is gently shaken with organic solvents, separate out the organic layer to that add ammonia solution, pink colour appears.

3. **Test for Flavonoids**

a) Shinoda test (Mg-HCl reduction test): To the alcoholic solution add few fragments of magnesium ribbon, add conc. HCl acid dropwise. Pink to red crimson red colour appears after few minutes.

b) Zn-HCl reduction test: To the test solution add a mixture of zinc dust and conc. HCl acid gives a red colour.

c) Ferric Chloride test: To the test solution with ferric chloride solution bluish green to black colour is produced.

4. **Test for Carbohydrates and Free Sugars in Glycosides**

Molisch's test: Treat the test solution with a few drops of molisch's reagent (Solution of -napthol in alcohol) and 2 ml of conc. H_2SO_4 acid slowly through the sides of the test tube, violet ring is formed at the junction of the two layers.

5. **Test for Tannins and Phenolics**

a) With gelatin solution: Treat the test solution with 1% gelatin solution containing sodium chloride, white precipitate appears.

b) With ferric chloride solution: Treat the test solution with few drops of freshly prepared neutral ferric chloride solution separately, bluish black colour appears.

c) Lead acetate test: To the test solution add few drops of 10% lead acetate solution, yellow precipitate appears.

d) Alcoholic HCl Test: To the test solution gently add alcoholic hydrochloric acid, red colour appears.

6. **Test for Saponins**

a) Froth test: Dilute aqueous extracts with distilled water separately to 20ml and shake in a graduated cylinder for 15 min, formation of 1cm layer of foam which is stable for 15 min takes place.

b) Haemolysis Test: sample is dissolved in physiological salt solution. To this 4% buffered equilibrated blood (pH 7.40) is added. Haemolysis of red blood cells occurs and can be noticed in the microscope.

7. **Test for Sterols**

a) Salkowanski test: When few drops of conc H_2SO_4 acid is added to the test solution in chloroform, shaken and allowed to stand, produces red colour in the chloroform layer.

b) Libermann- Burchard's test: The test solution in chloroform is treated with few drops of acetic anhydride and conc. H_2SO_4 acid is added form the sides of the test tube, it shows a brown ring at the junction of the two layers and the upper layer turns green.

8. **Test for Proteins and Amino Acids**

a) Millon's test: When proteins and amino acids are treated with millon's reagent, white precipitate appears which turns red upon gentle heating.

b) Ninhydrin test: Amino acids and proteins when boiled with 0.25 ml solution of ninhydrin reagent (indane-1, 2, 3 trionehydrate) violet colour appear.

c) Biuret test: When test solution is treated with biuret reagent, blue colour appears.

TABLE 10 Qualitative Chemical Investigation of Crude Drug Extract

	TEST	Amla				Baheda				Jamun,			
	Extracts	Pet-ether	Chloroform	Alcohol	Aqueous	Pet- ether	Chloroform	Alcohol	Aqueous	Pet- ether	Chloroform	Alcohol	Aqueous
1.	**Test for steroids** Salkowski												
a)	test Liebermann-burchard	+	-	-	-	-	+	-	-	+	+	-	-
b)	test Liebermann reaction	+	+	-	-	+	+	-	-	+	+	-	-
c)		+	+	-	-	+	+	-	-	+	+	-	-
2.	**Test for steroidal glycosides**	-	-	-	-	-	-	-	-	+	-	-	-
3.	**Test for triterpenoids**												

a)	Salkowski test Liebermann-	+	-	-	-	-	+	-	-	+	+	-	-
b)	burchard test	+	+	-	-	+	+	-	-	+	+	-	-
4.	**Test for glycosides**												
a)	Legal test	-	-	-	-	-	-	-	-	-	-	-	-
b)	Keller killani test Modified	-	-	-	-	-	-	-	-	-	-	-	-
c)	Borntrager test	-	-	+	+	-	-	+	+	-	+	+	+
5.	**Test for saponins** Foam test												
a)	Haemolysis test	-	-	-	-	-	-	-	-	-	-	-	-
b)		-	-	-	-	-	-	-	-	-	-	-	-
6.	**Test for carbohydrates**												
a)	Molisch's test Barfoed's test	-	-	+	+	-	-	+	+	-	-	+	+
b)	Benedicts test	-	-	-	-	-	-	-	-	-	-	-	-
c)	Fehling solution test	-	-	+	+	-	-	-	-	-	-	+	+
d)		-	-	+	+	-	-	+	+	-	-	+	+
7.	**Test for alkaloids**												
a)	Mayer's reagent test	-	-	-	-	-	-	-	-	+	+	+	+
b)	Dragondroff's reagent test	-	-	-	-	-	-	-	-	+	+	+	+
c)	Hager's reagent test	-	-	-	-	-	-	-	-	+	+	+	+
d)	Wagner reagent test	-	-	-	-	-	-	-	-	+	+	+	+
8.	**Test for Flavonoids**												
a)	Shinoda test	-	-	+	+	-	-	+	+	-	-	-	-
b)	Zinc/Hcl reduction test	-	-	+	+	-	-	+	+	-	-	-	-
9.	**Test for tannins**												
a)	5% Ferric chloride test	-	-	+	+	-	-	+	+	-	-	-	-
b)	Lead acetate test Potassium	-	-	-	+	-	-	+	+	-	-	-	-
c)	dichromate test	-	-	-	-	-	-	+	+	-	-	-	-
10.	**Test for proteins**												
a)	Biuret test	-	-	-	-	-	-	-	-	-	-	-	-
b)	Million reagent test	-	-	-	-	-	-	-	-	-	-	-	-
11.	**Test for amino acids**	-	-	-	-	-	-	-	-	-	-	-	-
a)	Ninhydrin test												

" +"(Positive) " -" (Negative)

	TEST	Karela				Nut Tree				Smilax china			
	Extracts	Pet- ether	Chloroform	Alcohol	Aqueous	Pet -ether	Chloroform	Alcohol	Aqueous	Pet -ether	Chloroform	Alcohol	Aqueous
1.	**Test for steroids** Salkowski												
a)	test Liebermann-burchard	-	-	-	-	+	-	-	-	-	-	-	-
b)	test Liebermann reaction	+	+	-	-	+	+	-	-	+	+	-	-
c)		+	+	-	-	+	-	-	-	+	-	-	-
2.	**Test for steroidal glycosides**	-	-	-	-	-	-	-	-	-	-	-	-
3.	**Test for triterpenoids**												
a)	Salkowski test Liebermann-	-	-	-	-	+	-	-	-	+	-	-	-
b)	burchard test	+	+	-	-	+	+	-	-	+	+	-	-
4.	**Test for glycosides**												
a)	Legal test	-	-	-	-	-	-	-	-	-	-	-	-
b)	Keller killani test Modified	-	-	-	-	-	-	-	-	-	-	-	-
c)	Borntrager test	-	-	+	+	-	-	-	-	-	-	-	-
5.	**Test for saponins** Foam test												
a)	Haemolysis test	-	-	-	-	-	-	-	-	-	-	-	-
b)		-	-	-	-	-	-	-	-	-	-	-	-

6.	**Test for carbohydrates**												
a)	Molisch's test Barfoed's test	-	-	+	+	-	-	-	-	-	-	+	+
b)	Benedicts test	-	-	-	-	-	-	-	-	-	-	-	-
c)	Fehling solution test	-	-	+	+	-	-	-	-	-	-	-	-
d)		-	-	+	+	-	-	-	-	-	-	+	+
7.	**Test for alkaloids**												
a)	Mayer's reagent test	-	-	-	-	-	-	-	-	-	-	-	-
b)	Dragondroff's reagent test	-	-	-	-	-	-	-	-	-	-	-	-
c)	Hager's reagent test	-	-	-	-	-	-	-	-	-	-	-	-
d)	Wagner reagent test	-	-	-	-	-	-	-	-	-	-	-	-
8.	**Test for Flavonoids**												
a)	Shinoda test	-	-	+	+	-	-	+	+	-	-	+	+
b)	Zinc/Hcl reduction test	-	-	+	+	-	-	+	+	-	-	+	+
9.	**Test for tannins**												
a)	5% Ferric chloride test	-	-	+	+	-	+	+	+	-	+	+	+
b)	Lead acetate test Potassium	-	-	+	+	-	+	+	+	-	+	+	+
c)	dichromate test	-	-	+	+	-	+	+	+	-	+	+	+
10.	**Test for proteins**												
a)	Biuret test	-	+	+	+	-	-	-	-	-	-	-	-
b)	Million reagent test	-	-	+	+	-	-	-	-	-	-	-	-
11.	**Test for amino acids**	-	-	+	+	-	-	-	-	-	-	-	-
a)	Ninhydrin test												

"+" (Positive) " -" (Negative)

V. Streptozotocin induced diabetic model

The animals were selected, weighed then marked for individual identification. Streptozotocin monohydrate was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg b.w. intraperitonially. After one hour of Streptozotocin administration the animals were given feed ad libitm and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic Phase[11]. The animals were kept under observation and after 48 h blood glucose was measured by gluco-meter[12]. The diabetic rats (glucose level > 300 mg/dl) were separated and divided into different groups for experimental study, each group contain six animals.

A. Oral glucose tolerance test (OGTT)

Fasted rats were divided into six groups of six rats each. Group I served as normal control and received distilled water with Tween 80. Groups II received standard drug Glimipride as an aqueous suspension at a dose of 600µg/kg body weight. Group III to VI received different extracts at a dose of 500mg/kg body weight as a fine tween 80 suspension. After 30min of extract administration, the rats of all groups were orally treated with 2g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 30 and 60 and 120 min after glucose loading. Blood glucose levels were measured immediately by using Gluco-meter[13].

B. Preparation of dose for dried extracts

The petroleum ether (60-80 ^{0}C), chloroform, alcohol and aqueous extracts (500 mg/kg b.w) were formulated as suspension in distilled water using Tween-80 as suspending agent since Tween-80 has negligible effect on normal blood glucose level. The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

C. Preparation of standard drugs

Glimipride was used as the reference standard drug for evaluating the antidiabetic activity which was made into suspension in distilled water using Tween-80 as a suspending agent. The strength of suspension was prepared according to 600µg/kg b.w.[14].

D. Estimation of blood glucose level

The Accu-chek® Active blood glucose strips (stored in refrigerator) taken out from the Container. The gluco-meter was calibrated as according to the specifications mentioned in the strips. The blood removed from the rat-tail vein, is immediately spread on the marked end of the strip. The strip is inserted in the gluco-meter & after few seconds the gluco-meter displayed the blood glucose level[15-19].

E. Body weight measurement

Body weight has been measured totally two times during the course of study period (i.e., on before Streptozotocin induction (initial values), 1st day and 7th day of the treatment period), using a weighing scale[14].

The above treatments were given for a period of 7 days both in diabetic and non-diabetic animals. In OGTT animals treatments were given for a single day with a single dose administration of extracts[20].

F. Statistical Analysis

The results of the study were subjected to one way analysis of variance followed by Dunnett's t-test for multiple comparisons. Values with $P < 0.05$ were considered significant[21].

VI. RESULTS AND DISCUSSION

In the present study, the effect of pet ether, chloroform, ethanol and aqueous extracts were studied for antidiabetic activity in Streptozotocin induced diabetic rats and oral glucose tolerance test and results are expressed as change in blood glucose level.

In Streptozotocin induced model, as expected administration of Streptozotocin led to elevation of fasting blood sugar (FBS), which was maintained over a period of study in diabetic control group and these rats were given 7 days of daily treatment with aqueous, ethanolic, chloroform and petroleum ether extracts of above mentioned plants. The results were comparable with reference standard Glimipride. There was a significant elevation in blood glucose in Streptozotocin induced diabetic control ($p<0.001$) rats when compare with normal control.

In OGTT model, by 30 min after starting the glucose tolerance test, the blood glucose concentration increased rapidly form its initial value as was evident from normal control, but the plant extracts fed groups prevented significantly glucose- induced hyperglycemia at 30min and 90 min as compare to that of normal control.

A. Amla

In Streptozotocin induced model, the aqueous and petroleum ether extracts were found to possess blood glucose lowering potential but the action was delayed. It could only produce the significant reduction in glucose effects only after 5 hours as compare to diabetic control. This delayed action may be due to poor absorption of the drug extracts.

In OGTT, Maximum glucose tolerance was observed in aqueous extract and minimum glucose tolerance was observed in chloroform extract in 90 minutes compared with the normal control.

B. Baheda

Ethanolic and chloroforn extract had led to a significant fall in the blood glucose level. Pet ether extract was non-significant for acute study but it gradually restored glucose level nearer to normal level in subsequent days. The effect of aqueous extract did not showed significant activity on prolonged treatment but showed significant ($P<0.01$) activity at 1 hr in acute study compared to diabetic control. Ethanolic extract had significantly reduced glucose level at 3 hr and significant reduction was maintained for another 4 hour in a day. In prolonged treatment, the effects of alcoholic extract were nearly equal to that reference drug Glimipride.

In OGTT, maximum glucose tolerance was observed in ethanolic extract and minimum glucose tolerance was observed in pet ether extract in 90 minutes compared with the normal control.

C. Jamun

Salacia chinensis petroleum ether extract showed significant blood glucose lowering effects in Streptozotocin induced rats on prolonged treatment whereas chloroform extract brought down glucose level till in 3 hours after the single dose of 500mg/kg. b.w. as compare to diabetic control.

In OGTT, maximum glucose tolerance was observed in petroleum ether extract and minimum glucose tolerance was observed in aqueous extract in 90 minutes compared with the normal control.

D. Karela

The single dose of ethanolic extract (300 mg/kg b.w.) has more significantly ($P<0.01$) reduced the blood glucose level as compare to diabetic control at 7th day of the study

. Chloroform extract (500 mg/kg b.w.) shown significant reduction of blood glucose after 1 hour whereas ethanolic extract shown the significant reduction at 3 hrs. Aqueous extract of the same plant could not reduce glucose level at sub acute level though it showed reduction of glucose level at 7 hr. as compare to diabetic control.

In OGTT, maximum glucose tolerance was observed in chloroform extract and minimum glucose

tolerance was observed in aqueous extract 90 minutes compared with the normal control.

E. Nut Tree

Ethanolic and aqueous extracts demonstrated significant blood glucose lowering effects (P<0.01) after single dose of 500mg/ kg.b.w. and multiple doses for prolonged treatment. . In acute & prolonged treatment, the effects of alcoholic extract were nearly equal to that reference drug Glimipride whereas aqueous extract shown significant reduction only in prolonged treatment.

In OGTT, maximum glucose tolerance was observed in ethanolic extract and minimum glucose tolerance was observed in petroleum ether extract in 90 minutes compared with the normal control.

F. Smilex China

The ethanolic and aqueous extracts were able to reduce blood glucose level significantly(P<0.01) as compare to diabetic control but Pet. Ether extract in prolonged treatment significantly reduced glucose level as compare to diabetic control.

In OGTT, Maximum glucose tolerance was observed in aqueous extract and minimum glucose tolerance was observed in Chloroform extract in 90 minutes compared with the normal control.

Oral treatment with standard hypoglycemic agent Glimipride 600µg/kg body weight also able to reduce the elevated blood glucose level towards the normal at 7th day of treatment.

Glimipride treated group (600µg/kg) also prevented significantly glucose induced hyperglycemia at 30 min and 90 min as compare to Normal control.

Plant extracts fed groups prevented significantly glucose-induced hyperglycemia at 30min and 90 min. as compare to that of normal control at 30 min and 90 min.

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