EFFECT OF HERBAL MEDICINE "DIABETO-CURE" ON CLINICAL AND BIOCHEMICAL PARAMETERS OF METABOLIC SYNDROME

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Abstract - Diabetes mellitus is the most important disease involving the endocrine pancreas. The secretion of the pancreas is digestive in function and the internal secretions plays a major role in the regulation of metabolism. Diabetes is characterized by symptoms like polyuria (increase in urine output), increase in thirst (polydipsia), loss of weight and polyphagia (increased appetite) as major symptoms of insulin deficiency. Beside these, hyperglycemia (increase in blood glucose concentration), glucosuria (presence of glucose in urine), ketonuria and ketonemia (increased concentration of ketone bodies in the blood and urine) and muscular weakness have also been reported. Long-term complications involve cardiovascular diseases (stroke and myocardial infarction), renal disease (diabetic nephropathy), and loss of nerve function (diabetic neuropathy) and proliferative scarring of the retina (diabetic retinopathy) The Aim of present research work is to, design and develop herbal formulation in the treatment of type 2 diabetes. Effect of Herbal Medicine on glycemic control in diabetic subjects, effect of Herbal Medicine on W/H Ratio and BMI, in diabetic subjects, effect of Herbal Medicine on HbA1c in diabetic subjects.

Key Words - Diabetes mellitus, Polyuria, Glucosuria.

I. INTRODUCTION

Diabetes:

Diabetes is a metabolic disorder characterized by chronically elevated blood glucose above the normal range. It is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. (WHO media centre) 347 million people worldwide have diabetes[1].

- In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar[2]
- More than 80% of diabetes deaths occur in low- and middle-income countries[3].
- WHO projects that diabetes will be the 7th leading cause of death in 2030.[4]
- Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type II diabetes.

Types of Diabetes

Greek and Roman physicians used the term "Diabetes" to refer to conditions that result in large urine volume and are classified it into two types [5].

- Diabetes mellitus in which the urine tasted sweet.
- Diabetes inspidus in which the urine tasted tasteless.

II. MATERIAL AND METHOD

Aluminum chloride colorimetric assay method[6]

The total flavonoid contents were measured with the aluminum chloride colorimetric assay Aqueous, Ethanolic, Petroleum ether, Benzene and Hydro-alcoholic extract and different dilution of standard solution of Quercetin (10-100µg/ml) were added to 10 ml volumetric flask containing 4 ml water. To the above mixture0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After six minutes 2 ml of 1M NaOH was added and than the total volume was made up to 10 ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Results are provided in table-1.Total flavonoidcontent of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample. By obtained the result it is assured that hydro alcoholic extract of both plants gives maximum % of yield and total flavonoid milligram Quercetin equivalent.

Hydro-alcoholic extraction of Plant powder[7]

In the present study whole plant parts were reduced to fine powder and were subjected to maceration in a 1 liter conical flask for 7 days at room temperature with alcohol (70%) water (30%). The flasks were securely plugged with adsorbent cotton and were shaken periodically till complete maceration. After the complete extraction, the solvent was distilled off and concentrated on water bath to a dry residue. This residue used as drug for present study.

Preliminary phytochemical investigation of extracts[8]

Qualitative chemical tests of hydro-alcoholic extracts of plant powder were conducted to identify the various phyto-constituents. The various tests and reagents used are given below and observations are recorded.

2.1 Tests for Carbohydrates

Molisch's test (general test)

To 2-3 ml test solution (T.S.), added few drops of α – naphthol solution in alcohol, shaken and added

concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing sugars

a) **Fehling's test:** 1 ml Fehling's A and 1ml Fehling's B solution was mixed and boiled for one minute. Added equal volume of T.S. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

b) **Benedict's test:** Equal volume of benedict's reagent and T.S. in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in T.S.

Tests for Monosaccharides

Benedict's test: Equal volume of Barfoed's reagent and T.S. were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Tests for Hexose sugar

Cobalt-chloride test: 3 ml of T.S. was mixed with 2 ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non- Reducing Sugars

a) T.S. dose not give response of Fehling's and Benedict's test.

b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

2.2 Tests for Proteins

Physico-chemical Characterization of drug powder:

All plants were powdered and mixed their different parts to form powder were evaluated for various organoleptic and morphological characteristics like nature, colour, odour, and taste. The observed characteristics were correlated with the literature to validate the authenticity of the crude drug under investigation. Moisture and volatile content were found to be less than 10% w/w indicating that individual plant material has been properly dried.

Extractive values were determined by maceration process using non-polar solvent petroleum ether (40-60ºC), benzene and polar solvent ethanol and water. The extractive values of plant powder have been found to be 13.80 to 19.17% w/w with polar solvents - ethanol and water and 12.30% w/w to 11.26% w/w with non polar solvent - petroleum ether (40-60ºC) and benzene. This indicates that both polar and non polar constituents were present in plants powder. Results are indicated in Table: 1.

Result of % of yield of different extract of selected plants: Hydro-alcohol extract showed maximum amount of yield hence for the study hydro-alcohol extract was prepared by maceration. The percent yield of extracts and physical nature of extracts were summarized in Table: 2.

Result of aluminum chloride assay:

To determine total Flavonoids contained in different extract of powder aluminum chloride assay was carried out. The results indicates that amongst all tested extract, hydro alcohol (70:30) extract of both plants showed higher amount of total flavonoids. Results are indicated in Table: 3.

Result of preliminary phytochemical investigation

Phytochemical investigation showed the presence of primary and secondary plant metabolites. Hydro alcoholic extract of plant powder revealed the presence of carbohydrates, proteins, amino acids, flavonoids, alkaloids, tannins, vitamin C and glycosides. Results of phytochemical investigation have been summarized in Table 4. Effect of plant powder and standard drug on the blood glucose levels in glucose loaded rats (OGTT):

Glucose at dose of 2 g/kg was orally administered, after the administration of different treatment. Blood glucose level was estimated at zero, 30, 60, 120 and 240 minutes after treatment. Percentage reduction in Blood glucose level in different treated groups after 240 minutes was measured and found to be 34.27, 15.21, 17.88 and 14.66% for glibenclamide, Drug extract at low dose 200 mg/kg and Drug extract at dose 400 mg/kg treated group respectively..Hypoglycemic effect in glucose treated model. Results of OGTT are given in Table 5 and Figure -1

Effect of plant powder and standard drug on the blood glucose levels in alloxan induced diabetic rats (Single day study)

Alloxan at dose of 60 mg/kg was i.p. administered, after the administration of different treatment. Blood glucose level was estimated at zero, 2, 4, 6 and 8 hours after treatment. Percentage reduction in Blood glucose level in different treated groups after 8 hours was measured and found to be 24.25, 16.40 and 21.79% for glibenclamide, Drug extract at low dose 200 mg/kg and Drug extract at dose 400 mg/kg treated group respectively. Results of alloxan induced diabetic rats (single day study) are given in Table- 6 and Figure- 2

Comparison of drug and placebo on various renal function and liver functions:

Comparison of drug and placebo on various renal functions and liver functions are given in Table-8.

Observation of side effects of drug in patients The observation of drug side effects have been noticed and recorded in 50 patients consumed drug. The data shows almost nil side effects of herbal preparation. Observations of side effects are given in Table-9

Table 1. Physico-chemical Characterization of drug powder

S.No.	Parameters	Observation
I	Physical Tests	
	Nature	Coarse Powder
	Color	Brown (Light Brick red)
	Odor	Characteristic
	Taste	No taste or slight Bitter
II	Extractive values	
	Aqueous	13.82%
	Alcohol	15.44%
	Benzene	12.30%
	Petroleum Ether	11.26%
	Hydro –Alcoholic (70:30)	19.17%
III	Loss on drying	11%
IV	Ash values	
	Total ash	12%
	Acid insoluble ash	3%
	Water soluble ash	1%

Table 2. Result of % of yield of different extract of selected plants

S.No.	Extracts	Nature of extract	Color of extract	% yield (w/w)
1	Aqueous	Semi solid Viscous	Light brown	11.75%
2	Alcoholic	Semi solid Viscous	Light brown	14.70%
3	Benzene	Semi solid Viscous	Light brown	10.50%
4	Petroleum Ether	Semi solid Viscous	Light brown	08.45%
5	Hydro-Alcoholic	Semi solid Viscous	Light brown	19.75%
Highest % of yield of extract obtained in hydro-alcoholic (70:30) extract of is 19.75%				

Table 3. Total Flavonoids contained in different extract aluminum chloride assay

S.No.	Extract solvent	Total Flavonoids Mg/QE/gm	Plants Extract
1	Aqueous	In Aqueous	125.84 ± 2.88
2	Alcoholic	In Alcoholic	132.01 ± 1.74
3	Benzene	In Benzene	105.17 ± 3.42
4	Pet. Ether	In Pet. Ether	96.28 ± 2.47
5	Hydro-alcoholic	In Hydro-alcoholic	168.5 ± 2.75

Table 4. Preliminary phytochemical investigation of hydro alcoholic extracts of plant extract

S.No.	NAME OF THE TEST	OBSERVATION
I	Test for Carbohydrates	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
	d. Barfoed's test	+
	e. Cobalt-Chloride test	+
	f. Test for non-reducing sugar	+
II	Test for Proteins	
	a. Biuret test	-
	b. Million's test	-
	c. Xanthoproteintets	-
	d. Test for protein containing sulphur	–
	e. Precipitation test	-
III	Test for steroids	
	a. Salkowski reaction	-
	b. Liberman- Burchard	-
	c. Liberman's reaction	-
IV	Test for amino acids	
	a. Ninhydrin test	+
	b. Test for tyrosine	+

	c. Test for tryptophan	+
V	Test for flavonoids a. Shinoda test b. Lead acetate test c. Alkaline solution test d. Ferric chloride test	 + + + +
VI	Test for alkaloids a. Dragendroff test b. Mayer's test c. Hager's test d. Wagner test	 + + + +
VII	Test for Tannins a. Lead acetate test b. 5% $FeCl_3$ test c. Gelatin solution d. Bromine water	 - - - -
VIII	Test for Vitamins a. Test for Vitamin A b. Test for Vitamin C c. Test for Vitamin D	 - + -
IX	Test for Glycosides a. Part a test b. Part B test c. Beljet's test d. Legal's test e. Keller killani test f. Libermann's test	 + + + + +
X	Test for Triterpenoids a. Salkowaski test b. Liebermann Burchardt test	 - -

Table 5. Effect of plant powder and standard drug on the blood glucose levels in glucose loaded rats (OGTT)

Group	0 Minute	30 Minutes	60 Minutes	120 Minutes	240 Minutes	Percentage reduction of blood glucose level after 4 hours
I	92.95 ± 0.789	92.66 ± 0.794	93.11 ± 0.599	91.83 ± 0.772	91.08 ± 0.802	-
II	92.95 ± 1.224	170.93 ± 1.397	174.21 ± 2.335	170.13 ± 1.388	141.43 ±3.549	-
III	93.65 ± 0.999	166.0 ± 3.038	158.55 ± 2.519	146.8 ± 2.682	110.46 ± 3.268	15.21%
IV	92.26 ± 1.175	162.73 ± 2.570	161.31 ± 2.688	152.56 ± 2.419	112.35 ± 2.839	17.88%
V	92.11 ± 1.045	154.75 ± 2.537	158.6 ± 2.604	148.08 ± 2.153	108.11 ± 3.500	14.66%

Group I- Only vehicle treated
Group II- Control only glucose (2000 mg/kg) treated
Group III- Glucose + Gilbenclamide 2.5 mg/kg
Group IV- Glucose + Drug extract low dose 200 mg/kg
Group V- Glucose + Drug extract- high dose 400 mg/kg

Table 6. Effect of plant powder and standard drug on the blood glucose levels in alloxan induced diabetic rats (Single day study)

Group	0 hours	2 hours	4 hours	6 hours	8 hours	Percentage reduction of blood glucose level after 8 hours
I	92.51 ± 1.17	92.7 ± 1.10	92.7 ± 1.00	93.3 ± 0.97	93.1 ± 0.95	-
II	274.8 ± 2.22	289 ± 2.40	296.8 ± 1.76	283.6 ± 1.56	275.8 ± 1.15	-
III	279.9 ± 1.62	243.9 ± 2.09	234.7 ± 1.95	220.3 ± 2.14	212.0 ± 1.55	24.25%

IV	278.5 ± 1.21	269.3 ± 0.85	254.9 ± 1.20	253.8 ± 1.55	232.8 ± 2.35	16.40%
V	283.1 ± 1.53	264.4 ± 2.09	251.8 ± 1.31	243.8 ± 1.40	221.4 ± 1.52	21.79%

Group I - Only vehicle treated
Group II- Control only alloxan (60 mg/kg) treated
Group III- Alloxan + Gilbenclamide 2.5 mg/kg
Group IV- Alloxan + Drug extract low dose 200 mg/kg
Group V- Alloxan + Drug extract- high dose 400 mg/kg

Table 7. Comparison of various parameters Using drug after 3 Month on diabetic and non diabetic human volunteers

Parameters		Drug				Placebo			
		Baseline	After 3 months	t	p	Baseline	After 3 months	t	p
		Mean±SE	Mean±SE			Mean±SE	Mean±SE		
BMI		24.68±0.64	26.44±0.62	2.18	<0.05	27.17±0.89	28.45±0.77	0.22	NS
W/H Ratio		0.96±0.01	0.96±0.01	-	-	0.95±0.01	0.96±0.01	1.00	NS
Pulse		80.08±0.91	80.76±0.61	0.64	NS	81.00±0.91	82.89±0.69	1.65	NS
BP	Systolic	136.15±3.30	126.42±1.51	2.66	<0.02	140.12±3.38	141.67±3.00	0.27	NS
	Diastolic	86.31±1.88	83.37±1.05	1.31	<0.2	85.40±2.16	88.94±1.37	1.47	NS
FBS		231.13±8.81	136.16±4.96	9.59	<0.001	247.35±5.81	272.50±4.73	3.29	<0.001
HbA_1c		8.40±0.30	6.37±0.10	6.51	<0.001	10.05±0.17	11.43±0.19	1.67	NS

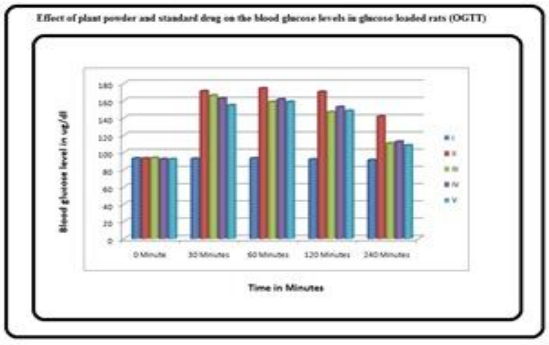
Table 8. Comparison of renal function and liver function Using drug after 3 Month on diabetic and non diabetic human volunteers

		Drug		Placebo	
Parameters		0 month	3 months	0 month	3 months
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Renal Function	B urea (mg/dl)	28.80±1.18	28.10±1.44	29.33±0.91	30.81±1.03
	S. Creatinine (mg/dl)	0.97±0.25	0.95±0.04	0.99±0.04	1.00±0.03
LFT	S. Bilirubin	0.76±0.19	0.75±0.19	0.70±1.5	0.71±0.07
	SGOT (IU/L)	24.50±1.21	23.90±1.01	25.15±1.06	25.75±1.35
	SGPT (IU/L)	25.29±1.27	26.71±2.86	24.07±0.86	24.67±1.02

Table 9. Observation of side effects of given drug

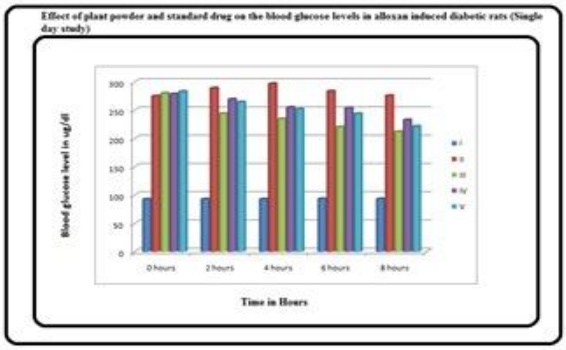
Side Effects	n (n= 50)
Poor Glycemic Control	2
Vomiting	0
Loss of appetite	0
Burning epigastrium	0
Liver dysfunction	0
Renal dysfunction	0

Figure 1. Effect of plant powder and standard drug on the blood glucose levels in glucose loaded rats (OGTT)



Group I -Only vehicle treated
Group II -Control only glucose (2000 mg/kg) treated
Group III-Glucose + Gilbenclamide 2.5 mg/kg
Group IV-Glucose+ Drug extract low dose 200 mg/kg
Group V-Glucose + Drug extract- high dose 400 mg/kg

Figure 2. Effect of plant powder and standard drug on the blood glucose levels in alloxan induced diabetic rats (Single day study)



Group I- Only vehicle treated
Group II-Control only alloxan (60 mg/kg) treated
Group III-Alloxan+ Gilbenclamide 2.5 mg/kg
Group IV-Alloxan+ Drug extract low dose 200 mg/kg
Group V-Alloxan + Drug extract- high dose 400 mg/kg

Figure 3. Comparison of fasting blood sugar in treatment and placebo groups

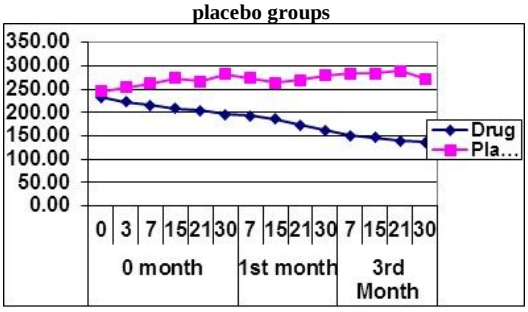
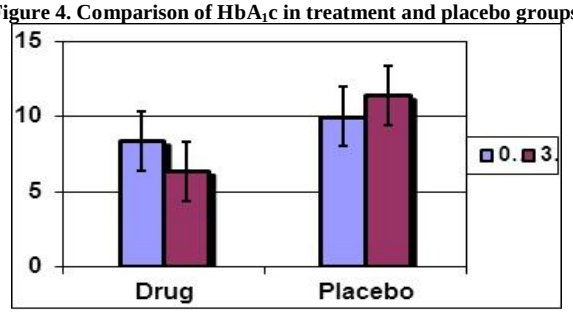


Figure 4. Comparison of HbA_1c in treatment and placebo groups



CONCLUSION

Diabetes mellitus is the most common endocrine disorder, affecting more than 300 million people worldwide. For the treatment, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects and are often too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. All the drugs discussed in the research exhibit significant clinical and pharmacological activity. The potency of herbal drugs is significant and they have negligible side effects than the synthetic anti diabetic drugs. In this research article an attempt has been made to focus on estimation of hypoglycemic activity of plants and maybe useful to the health professionals, scientists and scholars working in the field of pharmacology and therapeutics to develop evidence based alternative medicine to cure different kinds of diabetes in man and animals. Isolation and identification of active constituents from the plants, preparation of standardized dose and dosage regimen can play a significant role in improving the hypoglycemic action.

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