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Development and Evaluation of Anti-Diabetic Potential of Polyherbal Formulations in Experimental Animals

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ABSTRACT

The current study used experimental animals to examine the polyherbal formulation's potential anti-diabetic effects. Using the wet granulation process, the polyherbal formulation in the form of granules was created. The produced granules underwent pharmaceutical evaluation. Beneficial effects of Polyherbal formulations were tested on diabetic rats induced with alloxan. Rats were given a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) for 72 hours to develop diabetes. Following 72 hours of alloxan therapy for 11 days, the anti-diabetic effects of a polyherbal formulation were examined in diabetic rats induced with alloxan at two dose levels: 200 mg/kg and 400 mg/kg. The usual medication was Glibencamide at a dosage of 4 mg/kg. In diabetic rats, the polyherbal mixture demonstrated a decrease in blood glucose levels. It also reduces the treated groups' levels of triglycerides and cholesterol. The polyherbal mixture contains alkaloids, glycosides, saponin tannins, amino acids, and other ingredients, according to preliminary testing. α-amylase activity was responsible for the inhibitory activity. Blood glucose, serum triglycerides, and cholesterol were significantly increased ($P < 0.01$) in diabetic control. PHF treatment resulted in a significant ($P < 0.05$) drop in blood glucose levels when compared to diabetes control. PHF dramatically ($P < 0.05$) lowered the increased blood triglyceride and cholesterol levels. The

outcome demonstrated the safety of the polyherbal formulation and its high level of anti-diabetic action.

Keywords: Polyherbal Formulation's, Wet Granulation Process, Glibencamide, Serum Triglycerides.

INTRODUCTION

Polyuria (frequent and copious urine production), Glycosuria (glucose in urine), and hyperglycemia (glucose rate on an empty stomach greater than 1.2 g/l in plasma blood and confirmed in at least two occasions) are the hallmarks of diabetes, a metabolic illness. Thickening of the capillary basement membrane, an increase in the matrix of the vessel wall, and cellular proliferation are widespread pathological changes that lead to vascular complications such as lumen narrowing, early atherosclerosis, glomerular capillary sclerosis, retinopathy, neuropathy, and peripheral vascular inefficiency. Diabetes also causes eye and kidney issues. One of the main causes of death and disability is diabetes. Nowadays, the treatment of diabetes involves the use of hypoglycaemics (insulin, biguanides, and sulphonamides), as well as hygienic practices, food, and exercise regimens **(Srivastava et *al,* 2018).** The mechanism of insulin release in normal pancreatic β-cells: The beta cells produce insulin in a relatively steady amount. Food releases insulin when it reaches absorbable glucose. It is the primary hormone that controls how most cells absorb glucose from the blood. Therefore, a major contributing factor to all types of diabetes mellitus is a lack of insulin or its receptors.

The signal for glucose conversion to glycogen for internal storage in the liver and muscle cells is regulated by insulin. When glucose levels fall, β-cells' production of insulin also declines, and the conversion of glycogen back to glucose also occurs in reverse. The primary hormone responsible for this is glucagon, which functions in opposition to insulin. When blood glucose levels rise above the renal threshold, part of the glucose is reabsorbed in the proximal renal tubule and remains in the urine (a condition known as glycosuria). This causes the urine's osmotic pressure to rise and prevents the kidneys from reabsorbing water, which leads to polyuria **(Barar, 2008; Walker and Whittlesea, 2008).**

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MATERIAL AND METHOD

Collection of powders

This powdered polyherbal mixture is intended to provide anti-diabetic effects. It is created by combining several plant parts with possible antidiabetic properties. The powdered medicinal plants that are needed to make polyherbal granules were gathered from a supplier of medicinal plants in Nanded, Maharashtra.

Animals

Wistar rats of both sexes, weighing 180 ± 10 g, were obtained from the Sudhakar Rao Naik Institute of Pharmacy located in Pusad, Maharashtra, India. The study was given the go-ahead by the Sudhakarrao Naik Institute of Pharmacy's Institute Animal Ethics Committee in Pusad, Maharashtra, India, and all animal experiments were conducted in compliance with the rules set forth by the Committee for the Purpose of Control and Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee authorized the experimental protocols. No. 1555/PO/a/11/CPCESA, IAEC Before the experiment began, the animals were given a week to get used to the lab environment.

Development of formulation

The convenience of the wet granulation process for small-scale preparations led to its selection. Each formula's powders and other ingredients were weighed, crushed, and screened through sieve number 80 in isolation. After combining all the materials, sieve number 80 was used one more time. The acacia gum solution was added gradually and combined with the substance. Following mixing, the powder mass was dried at 35°C in a vacuum dryer before being filtered through sieve number 18 to extract the granules. Following drying, the granules were once again filtered through sieve number 18 to get rid of larger granules before being kept in desiccators **(Ghiware *et al,* 2010).**

Table 1: Ingredients and Quantity Taken

Sr. No.	Plant Name	Part of Plant	Quantity Taken for 10 gm		
			PHF1	PHF2	PHF3
1	*Gymnema Sylvestre*	Leaves	3	0.4	2
2	*Pterocarpus Marsupium*	Heartwood	1	1	1
3	*Trigonella Foenum Graecum*	Seed	1.4	1.4	1

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4	*Momordica Charantia*	Seed	1	1	0.6
5	*Syzygium Cumini*	Seed	1.5	2	3
6	*Musa Paradisiaca*	Peel	0.6	3	0.4
7	*Swertia Chirata*	Leaves	1	0.2	1
8	*Tinospora Cordifolia*	Leaves	0.6	0.4	0.4
9	*Aegle Marmelose*	Leaves	0.1	0.4	0.4
10	*Plantago Ovata* (Disintegrant)	Husk	0.1	0.1	0.1
11	*Curcuma Longa* (Preservative)	Rhizome	0.1	0.1	0.1
12	Sodium Chloride Salt (Lubricant)		0.1	0.1	0.1
13	Gum Acacia (Binder)		0.1	0.1	0.1

Phytochemical evaluation

The presence or lack of specific chemical compounds in a plant is ascertained using a battery of tests. Standard protocols were followed when conducting chemical tests in order to determine the preliminary phytochemical screening by referral methodology (**Trease and Evans, 1989; Kokate, 1986;)**

Preparation: - The PHF (2000 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to phytochemical tests.

PHARMACEUTICAL EVALUATION (Aulton, 1996; Lachman et al., 1987)

Angle of repose: Determined by using the funnel method using **(tan □ = h/r)** formula.

Loose bulk density (LBD): Determined by pouring a weighed quantity of granules into a graduated cylinder and measuring the volume and weight.

LBD = Weight of the powder / volume of the packing

Tapped bulk density (TBD): Determined by placing a graduated cylinder, containing a known mass of granules.

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TBD = Weight of the powder / volume of the tapped packing

Hausner's ratio: It is the measurement of frictional resistance to the drug. The ideal range should be 1.2- 1.5. It is determined by using the following formula:

Hausner's ratio= TBD / LBD

Compressibility index: The Compressibility index of the blends was determined by the Carr's compressibility index.

Compressibility index (%) = (TBD-LBD) X 100 / TBD

RESULTS

Preliminary identification test

Preliminary identification test showed that the presence of alkaloids, glycosides, tannins, saponin,amino acids, carbohydrates and flavonoids.

Table 2: preliminary identification test

Name of the chemical test	Observation
Mayer' s test	+
Wagner's test	+
Ninhydrin test	+
Molish' s test	+
Benedict' s test	+
. Bontrager's test	+
Legal's test	+
Foam test	+
Shinoda test:	+
Alkaline reagent test:	+
Ferric chloride test	+

Pharmaceutical evaluation

The bulk density and tapped density of polyherbal granules are demonstrated by the pharmaceutical evaluation. The granules' % compressibility, Hausner's ratio, and angle of repose were all quite satisfactory.

Table 3: Pharmaceutical evaluation

FORMULATIONS	BULK DENSITY	TAPPED DENSITY	CARR'S INDEX	HAUSSNER'S RATIO	ANGLE OF REPOSE
PHF 1	0.45 ±0.005	0.50 ±0.017	6.7	1.11 ±0.028	32.21º ±0.46
PHF 2	0.46 ±0.01	0.50 ±0.015	6.7	1.09 ±0.036	33.28º ±0.61
PHF 3	0.46 ±0.005	0.51 ±0.011	6	1.10 ±0.040	33.01º ±0.69

PHARMACOLOGICAL EVALUATION

Evaluation of in vitro α-amylase inhibitory activity

The % inhibitory activity against α-amylase increased in a dose-dependent manner. PHF 1 demonstrated a % inhibition of 30.82 ±0.476 at 200 µg/ml and 70.60 ±0.54 at 1000 µg/ml. The IC50 value obtained from the PHF1 was 550.56±78.36µg/ml. PHF 2 demonstrated a percentage inhibition of 35.22±0.485 at a concentration of 2000µg/ml and 68.8±0.918 at a concentration of 1000µg/ml. 520.8±1.64µg/ml was the IC50 value that the PHF2 yielded. The % inhibition for PHF 3 at 200µg/ml was 42.9±4.510, while at 1000µg/ml it was 66.9±3.408. The IC50 value obtained from the PHF 3 was 490.63±1.46µg/ml. The standard medication acarbose was found to have an IC50 value of 568.27±2.86µg/ml.

Table 4: *in-vitro* α-amylase inhibition

	% Inhibition			
Concentration µg/ml	PHF 1	PHF 2	PHF 3	Acarbose
200	30.82±0.476	35.22±0.485	42.9±4.510	37.39±0.457
400	46.78±0.415	39.67.1±0.915	48.02±4.656	46.01±0.488
600	57.12±0.455	55.5 ± 0.5067	61.32±2.321	61.64±0.347

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800	65.5±0.4857	60.9 ± 1.908	64.93±1.64	70.7±0.251
1000	70.60±0.54	68.8 ± 0.918	66.9±3.408	83.87±0.55
IC50 µg/ml	550.56±78.36	520.8±1.64	490.63±1.46	568.27±2.86

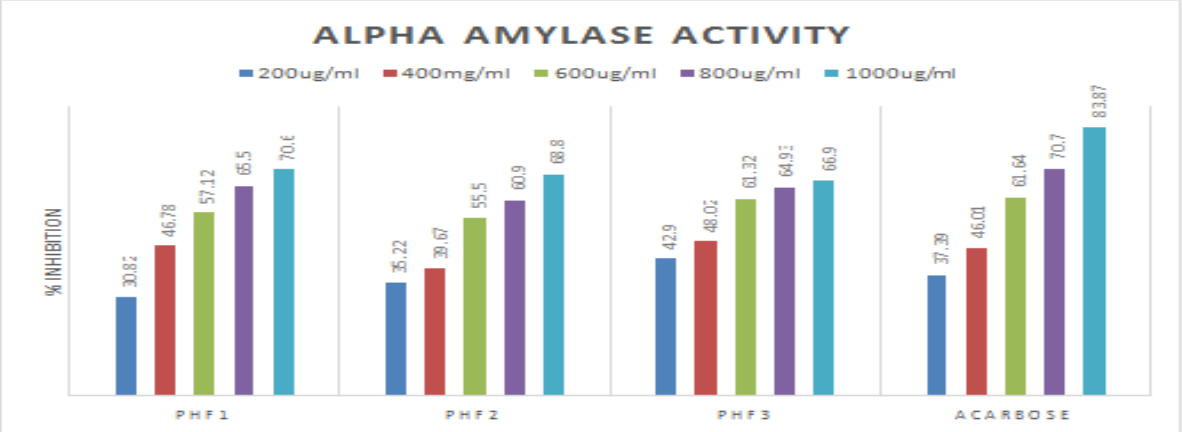

Figure 1: in-vitro α-amylase inhibition

Anti- diabetic activity of polyherbal formulation in alloxan induced diabetic rats A] Estimation of blood glucose

The normal control group's blood glucose level did not change. When compared to the normal control group, the diabetes control group's blood glucose level increased significantly till day 11 ($p<0.001$). The blood glucose levels of the groups treated with the polyherbal formulation at doses of 400 mg/dl and 200 mg/dl significantly decreased at all time intervals ($p<0.001$), and its hypoglycemic activity was compared to that of the conventional medication Glibenclamide.

Table 5: Effect of polyherbal formulation on glucose level

Blood Glucose Level (mg/dl) Values are Mean ±SEM, N = 6				
Groups	Day 0	Day 3	Day 7	Day 11
Normal Control	91.42 ±6.56	97.04 ±11.80	99.55 ±2.368	96.32 ±7.12
Control	265.82 ±1.316#	276.58 ±0.937#	280.46 ±4.314#	285.11 ±5.182#
Glibenclamide	258.39±3.36	248.52 ±4.49*	229.10±8.34**	217.47 ±4.31***
PHF (400 mg/dl)	252.85±5.524	245.48±5.6189*	233.90±4.905***	223.56±4.5008***

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PHF (200 mg/dl)	245.01±0.5**	235.24±0.83***	221.51±3.80***	206.19±5.63***

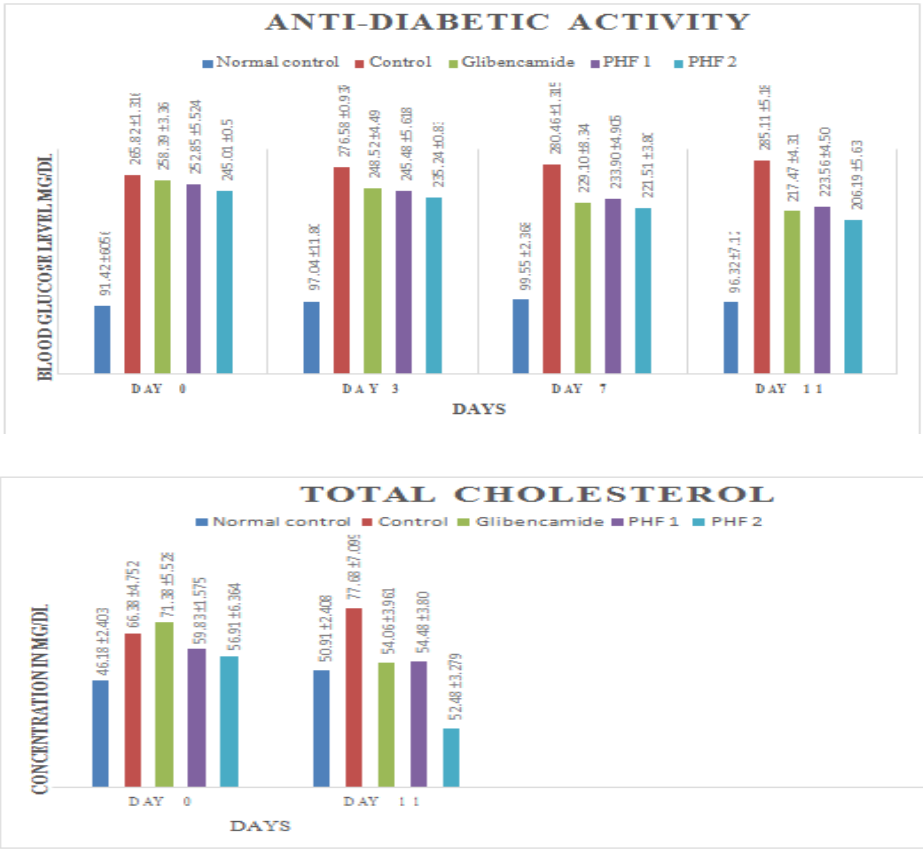

Figure2: Effect of polyherbal formulation on glucose level

ESTIMATION OF LIPID PROFILE

Estimation of total cholesterol

Over the course of the investigation, the cholesterol level in the normal control group stayed almost constant. On the eleventh day, the blood cholesterol level in the diabetic control group was significantly higher ($p<0.05$) than in the normal control group. When compared to the diabetic control group, the cholesterol levels were significantly ($p<0.05$) lower in the groups treated with polyherbal remedies and Glibenclamide.

Table 6: Effect of polyherbal formulation on cholesterol level

Blood Cholesterol Levels mg/dl, Values are Mean ± SEM, N=6					
Day	Normal Control	Diabetic Control	Glibenclamide	Polyherbal treated (400 mg/dl)	Polyherbal treated (200 mg/dl)
0 day	46.18 ±2.403	66.38±4.752*	71.38 ±5.528	59.83 ±1.575	56.91±6.364*
11 day	50.91 ±2.408	77.68 ±7.099	54.06 ±3.961*	54.48 ±3.80*	52.48 ±3.279*

Figure3: Effect of polyherbal formulation on cholesterol level

Estimation of triglycerides

Throughout the course of the trial, the triglyceride level in the normal control group stayed quite consistent. On the eleventh day, the blood triglyceride level in the diabetic control group was significantly higher ($p<0.01$) than in the normal control group. Triglyceride levels were significantly ($p<0.05$) lower in the groups treated with polyherbal remedies and Glibencamide than in the diabetes control group.

Table 7: Effect of polyherbal formulation on triglyceride level

Blood Triglyceride Levels mg/dl, Values are Mean ± SEM, N=6					
Day	Normal Control	Diabetic Control	Glibencamide	Polyherbal treated (400 mg/dl)	Polyherbal treated (200 mg/dl)
0 day	76.11 ±9.488	114.43 ±7.306*	125.45 ±3.279	133.85 ±4.725	132.27±7.219
11 day	77.68 ±7.099	132.27±7.219**	99.21 ±8.447*	91.33 ±5.681**	93.21±1.160*

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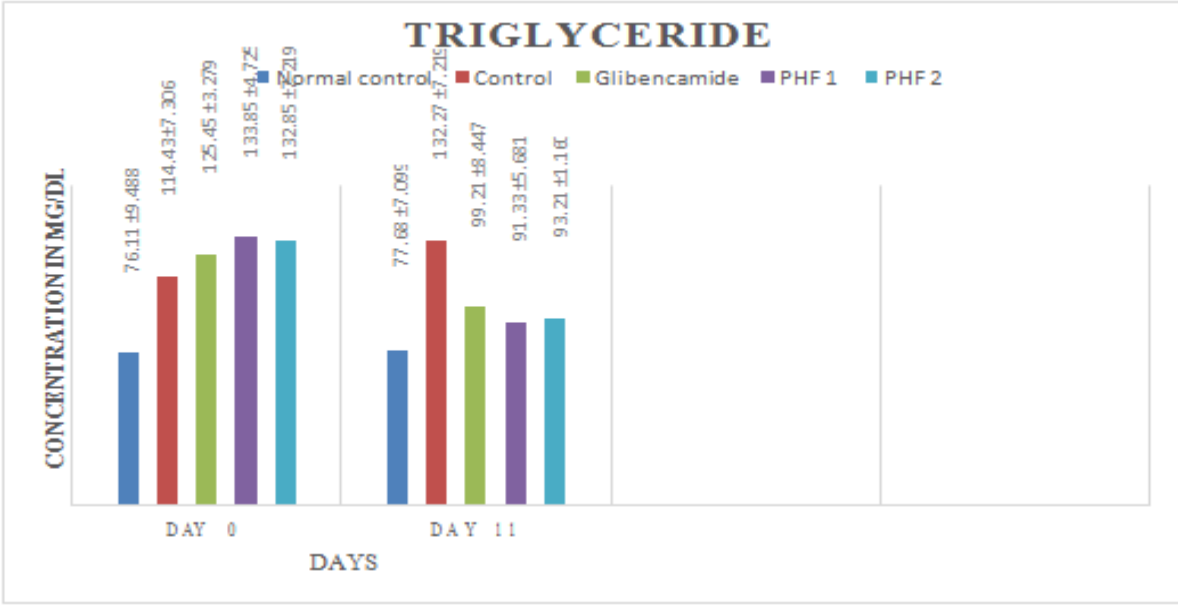

Figure 4: Effect of polyherbal formulation on triglyceride level

DISCUSSION

Diabetes is classified by the WHO as a "metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in the metabolism of carbohydrates, fats, and proteins resulting from defects in insulin action or secretion, or both"**(WHO Consultant, 1998).** Long-term medication side effects including organ dysfunction and failure, including kidney, nerves, heart, and gastrointestinal tract, are consequences of diabetes. It is the most prevalent endocrine condition globally, with an incidence ranging from 1 to 8% **(Haller *et al*, 1996).** The lack of a safe, effective medication makes treating diabetes difficult and preventive in terms of histology, biochemistry, and clinical outcomes. Conversely, herbal medications have become more well-known globally, mostly because they are safer, have less side effects, and consistently lower blood sugar levels. The worry over the side effects and expense of long-term synthetic drug use for diabetic patients has led to a rise in the use of herbal therapy in wealthy nations. Numerous phytoconstituents are beneficial in the management of diabetes. Alkaloids, glycosides, peptidoglycan, steroids, terpenoids, amino acids, guanidine, and inorganic ions are a few of them. An ethnobotanical survey indicates that over 800 plants have the ability to prevent diabetes **(Alarcon *et al*, 1998).**

Gymnemic acid, also known as gymnenin or saponin, decreases blood sugar levels by inhibiting glucose receptors and promoting the release of endogenous insulin

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reserves. Gymnemic acid increases insulin-dependent cell permeability in normal rats and decreases cholesterol in hypertensive rats. Gurmarin shortens the tongue's perception of sweetness over a period of 15 to 24 hours **(Shigematsu *et al.*, 2001; Reddy *et al.*, 1989; Yackzan, 1966).**

From the Syzygium cumini (L.) seed, phytoconstituent extracted and identified the purported antidiabetic component. They separated mycaminose from SC seed extract and examined its anti-diabetic properties in diabetic rats that had been given alloxan. They stated that mycaminose showed notable action **(Kumar *et al.*, 2008).** In M. charantia, charantin is a common cucurbitane-type triterpenoid and a possible antidiabetic agent **(Krawinkel and Keding, 2006; Patel *et al.*, 2010).**

In this study we illustrate about diabetes mellitus and its types, causes, sign and symptoms, complications, pathophysiology, diabetic medication, diabetic treatment, herbal diabetic cure, advantages of herbal medicines over allopathy and herbal formulations.

This study's primary goal is to provide an overview of antidiabetic action using the most popular model among researchers. We discovered that the animal model—diabetes produced by alloxan—is more frequently utilized since it requires a lower dose and produces results at a certain time. We recommend using both in-vivo and in-vitro models for optimal outcomes. The purpose of this study was to evaluate the anti-diabetic properties of a polyherbal formulation (PHF) made up of ten different herbs. Ten herbs are combined to create a polyherbal mixture that inhibits α-amylase activity, demonstrating antidiabetic efficacy. The significant amount of antidiabetic efficacy is demonstrated by the polyherbal formulation.

CONCLUSION

Rats with diabetes were able to significantly lower their blood glucose levels, which is evidence of the formulation's antidiabetic effectiveness. This finding suggests that the combination of herbal plants in polyherbal formulations has a notable antidiabetic impact. This might be the result of diverse active principle kinds from different plants, each of which could have a unique mode of action. As a result, the combination can be advantageous. The polyherbal formulation is thought to be a secure adjunctive treatment for the long-term and successful management of diabetes patients. In summary, the study introduces Polyherbal formulation as a novel approach to achieve antidiabetic effects. Therefore, it could be a useful addition to the current arsenal of anti-diabetic medications and aid in preventing complications from diabetes.

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